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Plate 1.—Showing the thymus gland of a 3-weeks-old puppy. Note that the cornua of the thymus extend upward on both sides to the thyroid glands. The cornua of the thymus extend upward to the thyroids on one side or on both sides very commonly in the cat and in some other animals, but rarely in the dog. (See page 355.)

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THE RESULTS OF THYMUS EXTIRPATION IN THE DOG

WITH A REVIEW OF THE EXPERIMENTAL LITERATURE ON THYMUS EXTIRPATION *

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BALTIMORE

INTRODUCTION

The experiments described in this paper were undertaken after reading reports of the results of thymus extirpation by Klose and Vogt and by Matti, in the belief that it was possible to produce in animals, by means of removal of the thymus gland, a condition closely related to, if not actually identical with, the rickets of human beings. The experiments did not result, however, as we had been led to anticipate. In the course of them we had occasion to read the experimental literature on thymus extirpation and became impressed with the inferior quality of much of the work done and the conflicting character of the results obtained. The reviews of the experimental literature failed to convey a proper conception of the character of the investigations which served as a basis for the ideas and theories so freely expressed in regard to thymus function, and did not indicate the paucity of actual knowledge concerning the effects of removal of the thymus gland.[1] Moreover, there was not to be found anywhere an adequate presentation of the work of Klose or of Matti, although the results of thymus extirpation announced by these two investigators had received a wide acceptance and had been instrumental in altering scientific opinion in

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1. The review of Wiesel,[2] admirable from so many standpoints, fails because it is uncritical. The review by Klose and Vogt[3] is incomplete and we believe gives a distorted conception of what has been accomplished by experimental extirpation of the thymus gland. Matti's critical review,[4] excellent in many respects, we feel gives a somewhat biased presentation of the subject.

2. Wiesel, J.: Pathologie des Thymus, Ergebn. d. allg. Path. u. path. Anat. [etc.] **15**:416, 1912.

3. Klose, H., and Vogt, H.: Klinik und Biologie der Thymusdrüse mit besonderer Berücksichtigung ihrer Beziehungen zu Knochen und Nervensystem, Beitr. z. klin. Chir. **69**:1, 1910.

4. Matti, H.: Physiologie und Pathologie der Thymusdrüse, Ergebn. d. inn. Med. u. Kinderh. **10**:1, 1913. Die Beziehungen der Thymus zum Morbus Basedowii, Berl. klin. Wchnschr. **51**:1310, 1914.

many quarters concerning the function and significance of the thymus.[5] Accordingly, we felt it desirable to include with the reports of our own experiments a critical study of the literature.

We have also added to the reports of our experiments a description of the development of the thymus in the higher mammals, in order to make plain the differences in the anatomic arrangement of the thymus in the species which most commonly have been used for thymectomy experiments, and to make clear the variations which often are found in different members of the same species; in other words, we have tried to show which animals are suitable and which unsuitable for thymectomy experiments, and to emphasize the fact that the thymus cannot always be extirpated completely in any species of animal and, perhaps, not nearly so often in any animal as is commonly thought.

In supplementing our own work with the historical and developmental studies mentioned, we have aimed to accomplish more than a mere orientation of ideas in regard to the results of experimental extirpation of the thymus; we have sought to indicate parts of the thymus problem which require further investigation, and lines of approach which offer possibilities of success. Moreover, we have endeavored to prevent the repetition of mistakes and useless experi-

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5. "Nachdem die Untersuchungen von Basch,[6] Klose[7] und Matti[8] den Beweis dafür erbracht hatten dass zwischen der Funktion des Thymus und dem wachsenden Knochensystem enge Beziehungen stehen, und nachdem die sorgfältigen Untersuchungen Mattis und Kloses gezeicht hatten, dass es sich dabei um ausgesprochene rachitische Veränderungen handelt, erhob sich von selbst die Frage, wie weit auch die menschliche Rachitis zu Thymuserkrankungen in Beziehung gesetzt werden dürfe."—(Aschoff,[8] p. 131.) ("Since the investigations of Basch, Klose and Matti have brought forward proof that a specific relationship exists between the function of the thymus and the growing skeleton, and since the painstaking investigations of Matti and Klose have shown that the changes in the skeleton consist in the most outspoken rachitic manifestations, the question naturally arises, to what extent the rachitis of human beings can be brought into relationship with diseases of the thymus.")

"Extirpation of the thymus in young animals is followed by marked defects in the development of the skeleton, and changes in the development of the sex organs. Some authors state that thymus extirpation causes a loss of calcium, and that a calcium retention results from feeding thymus, but the results quoted are not at all in harmony. It is certain, however, that deficiency in the thymus causes a defect in ossification. Also there occurs a period of adiposity, followed by cachexia with hyperplasia of the lymphatic tissues, thyroid, pancreas, ovaries and testicles (Klose and Vogt)."—(Wells,[9] p. 558.)

6. Basch, K.: Ueber Ausschaltung der Thymusdrüse, Verhandl d. Versamml. d. Gesellsch. f. Kinderh., Deutsch. Naturf. u. Aerzte, 1902, Wiesbaden, 1903, p. 239.

7. Klose, H.: Ueber Thymusexstirpation und ihre Folgen, Verhandl. d. deutsch. Gesellsch. f. Chir., Berl. **39**:379, 1910. Also: Arch. f. klin. Chir. **92**:1125, 1910.

8. Aschoff, L.: Ueber die Regeneration des Thymus, Tr. Internat. Cong. Med., 1913, Lond., 1914, Sect. 3, Gen. Path. & Path Anat., Part 2, p. 131.

9. Wells, H. G.: Chemical Pathology, Ed. 2, Philadelphia and London, W. B. Saunders Co., 1914, p. 606.

ments, and to emphasize the necessity of a radical change in the experimental methods employed, a change which is absolutely essential if it is ever to be hoped that a final settlement of the question of the effects of thymectomy can be achieved.

In conclusion, we wish to call attention to the fact that the scope of this paper is limited to the consideration of the removal of the thymus gland and its effects in animals. For a discussion of the much disputed question of the histogenesis of the thymus, of its pathology and the rôle which the thymus plays in clinical medicine, and also of the effects produced on the thymus by the removal of the various endocrine glands, the reader must search elsewhere.

THE DEVELOPMENT OF THE THYMUS GLAND IN THE HIGHER MAMMALS

In the lower vertebrates, which have numerous pharyngeal pouches, the thymus is formed from several pouches in series, but in the higher vertebrates with only five pharyngeal pouches altogether, the thymus is formed from only one or, at most, two. Furthermore, the thymus takes origin from dorsal diverticula of the pharyngeal pouches in the lower vertebrates but from ventral diverticula in the higher vertebrates, so that it is not, strictly speaking, the same organ in the entire vertebrate series when considered from the standpoint of its development.

The higher mammals may be divided into three groups, according to the situation of the thymus (Hammar).[9a] In the first group the thymus lies chiefly or entirely in the thorax; in the second group it lies in both thorax and neck; and in the third group it lies in the neck alone.

The thymus of most of the higher vertebrates (Fig. 1) is derived from the third pharyngeal pouch alone or from the third and fourth pouches, and is therefore purely entodermal. The thymus of the swine (Zotterman,[10] Badertscher[11]) and of the mole (Schaffer and Rabl[12]), on the other hand, is said to spring not only from the third pharyngeal pouch but also from the ductus precervicalis medialis, a derivative of the ectoderm, and the thymus of the guinea-pig (Maximow,[13] Ruben[14]) also is thought to have a two-fold origin, from entoderm and from the ectoderm of the vesicula cervicalis.

9a. Hammar, J. A.: Zur Histogenese und Involution der Thymusdrüse, Anat. Anz. **27**:23, 41, 1905.

10. Zotterman, Agne: Die Schweinethymus als eine Thymus ectoentodermalis, Anat. Anz. **38**:514, 1911.

11. Badertscher, J. A.: The Development of the Thymus in the Pig, Am. J. Anat. **17**:437, 1914.

12. Schaffer, J., and Rabl, H.: Das thyreo-thymische System des Maulwurfs und der Spitzmaus, Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Kl., Wien. **118**:217, 1909.

13. Maximow, A.: Untersuchungen über Blut und Bindegewebe. II. Ueber die Histogenese der Thymus bei Säugetieren, Arch. f. mikr. Anat. **74**:525, 1909.

14. Ruben, R.: Zur Embryologie der Thymus und der Parathyreoidea beim Meerschweinchen, Anat. Anz. **39**:571, 1911.

The mammalian thymus appears very early (in the human embryo of 5 mm.) as a cylindrical outgrowth in a ventral and mesial direction from the ventral diverticulum of the third pharyngeal pouch. Simultaneously with the appearance of this anlage, called thymus III, there occurs a proliferation of the epithelium of the dorsal diverticulum and adjacent parts of the pouch which soon undergoes histologic differentiation into the larger of the two parathyroids, parathyroid III.

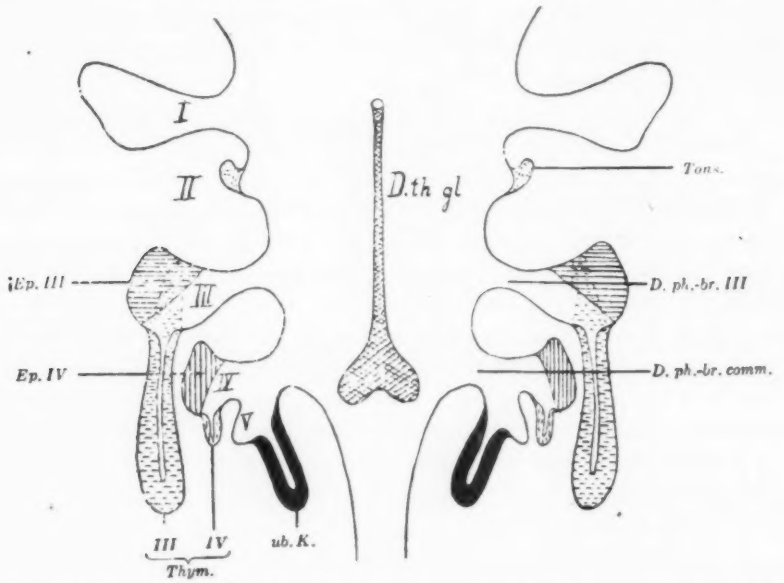

Fig. 1.—Diagram illustrating the development of the thymus in the higher mammals.

From Keibel and Mall, Manual of Human Embryology **2**:461. Figure 324.—Schema of the bronchiogenic derivatives in man, adapted from the schemata of Groschuff and Kohn. *D. ph.-br. III,* ductus pharyngobranchialis of the third pharyngeal pouch; *D. ph.-br. comm.,* ductus pharyngobranchialis communis of the fourth and fifth pouches (of the caudal pharyngeal pouch complex); *D. th.-gl.,* ductus thyreoglossus; *Ep. III and IV,* epithelial bodies of the third and fourth pouches; *Thym. III and IV,* thymus anlagen of the third and fourth pouches; *Tons.,* tonsil; *ub. K.,* ultimobranchial body.

While thymus III and parathyroid III are forming from the ventral and dorsal diverticula of the pouch, the mesial portion, that part of it lying between these structures and the pharynx, called the ductus pharyngobranchialis, narrows, atrophies and disappears altogether with the result that thymus III and parathyroid III, freed from their connection with the pharynx, become independent structures. The thymus anlage elongates and is now termed the thymus cord.

In the species constituting the first group, of which man and the dog are representative, the caudal end of the thymus cord thickens

and the cranial end becomes thinner. The entire cord then begins a migration in a caudal direction which is associated with that of the great vessels and heart; the thick caudal portion moves caudally faster than the thin cranial portion and the latter becomes a thin strand. The caudal end enters the thorax and, joining its fellow of the opposite side, forms the thoracic thymus. The thin cranial portion of the cord atrophies and disappears entirely, except the lowermost part, which persists as a continuation of the thoracic thymus into the base of the neck, forming the cervical process, or cornu, of the completely developed gland. Parathyroid III, which remains connected with the upper end of the thymus cord, is carried caudally with the latter, but halts near the lower end of the thyroid anlage, assuming its permanent position near the lower pole of the thyroid.

In the second group of animals, having both "neck" and "breast" thymus, of which the hoofed animals are examples, the caudal portion of the thymus cord enlarges and migrates into the thorax to form the thoracic thymus, exactly as has been described for the animals of the first group, but the remainder of the cord does not atrophy and disappear; indeed, the cranial end of it hypertrophies, forming an enlargement, known as the head of the thymus, and in certain members of the group, at least (ox, horse, goat and swine), it extends in a cranial direction with the growth of the neck, or, according to some embryologists, is caught in the loop of the hypoglossal nerve, and drawn to a position opposite the bifurcation of the great vessels, well above its point of origin. Shortly before birth, or during the first year, the stem of the thymus, connecting cervical and thoracic portions, disappears, so that the two parts of the gland become separate.

In the guinea-pig, which forms the third group, the thymus develops from the whole of the third pharyngeal pouch, with the exception of those parts which form parathyroid III and the neck. When set free, together with parathyroid III, from its connection with the pharynx through atrophy of the neck of the pouch, the thymus anlage does not elongate, as in those animals destined to have a thoracic thymus, but, assuming a rounded or oval form, remains essentially in its original position. According to Ruben,[14] the loop of the hypoglossal nerve cuts in between parathyroid III and the thymus mass, separating the former from the latter without carrying the latter upward. A portion of the ectodermal precervical vesicle early becomes incorporated.

In certain species of animals a thymus anlage—termed thymus IV or thymus metamere IV—first identified by Groschuff,[15] develops at

15. Groschuff, K.: Bemerkungen zu der vorläufigen Mitteilung von Jacoby: Ueber die Entwickelung der Nebendrüse der Schildrüse und der Carotiden drüsen (Anat. Anz. **12**:152); Anat. Anz. **12**:497, 1896. Ueber das Vorkommen eines Thymussegmentes der vierten Kiementasche beim Menschen, Anat. Anz. **17**:161, 1900.

the fourth pouch, together with the parathyroid of that pouch, parathyroid IV, exactly in the manner described for the development of thymus and parathyroid from the third pouch. When the neck of the fourth pouch—the ductus pharyngobranchialis communis—atrophies and disappears, like its fellow of the third pouch, thymus IV and parathyroid IV in their turn are set free. In company with the ultimobranchial body,[16] they wander in a medial and ventral direction to the thyroid anlage and come to rest behind the thyroid. If fusion occurs between these structures and the thyroid, parathyroid IV and thymus IV become imbedded in, or completely surrounded by, the substance of the thyroid. Parathyroid IV is not constant in all the higher mammals and thymus IV occurs as a regular development in none of them (Groschuff[15]), but is fairly regularly found in some species and occasionally found in others, as will be explained later.

The development of the thymus in the different species of the higher mammals does not always take place in the manner described as normal for the group to which the species in question belongs; in other words, variations in the distribution of the thymus in any given species are not infrequent. These variations can always be understood from a knowledge of the general principles of the development of the thymus and are to be explained like most other variations from normal development as arrests or cessations at different stages of the full developmental process.

The developmental process is most advanced, that is has progressed furthest from its starting point, in the higher mammals included under Group I, i. e., in man and the dog; it is most rudimentary, in fact, has changed scarcely at all from the early embryonic condition in the guinea-pig; in the hoofed animals, representative of Group II, it remains at an intermediate stage. The fully developed thymus in this latter group is, in its essential characteristics, the fully descended but unatrophied fetal thymus of man and the dog, projected into post fetal life.

The persistence of the cervical portion of the thymus in its entirety is, as already stated, normal for the hoofed animals, is not uncommon in the cat and occasionally is found in man and the dog, and doubtless also in the rabbit and rat. Harman[16a] has reported a case in a full term human fetus in which the cervical portion of the thymus not only had failed to atrophy, but extended upward to the region between hyoid bone and thyroid and terminated in an enlargement, exactly

16. The ultimobranchial body is the remnant of the fifth pharyngeal pouch.

16a. Harman, N. B.: Socia thymi cervicalis and thymus accessorius, J. Anat. & Physiol. **36**:47, 1901. Also: Brit. M. J. **2**:1072, 1901.

duplicating a condition found in the hoofed animals. Bien[17] has described two similar cases in human beings. The persistence of the caudal portions only of the cervical processes of the thymus or of the cervical process on one side alone, is, however, far more common — except, of course, in the hoofed animals — than the persistence of both cervical portions in their entirety. In the dog, for example, it is not unusual to find one, sometimes both cornua of the thymus prolonged upward, often as delicate processes or mere filaments, along the sheaths of the great vessels for variable distances toward or actually to the lower poles of the thyroid. Analogous conditions are exceedingly common in the cat and, we presume, in the rabbit and rat.

In certain species the upper end of the cervical strand of the thymus not infrequently becomes separated from the rest, and persists in the form of a small island or islands of thymus tissue near the lower pole of the thyroid and in close proximity to, or in contact with, the lower of the two parathyroids, parathyroid III. In the cat small masses of thymus tissue having this origin are commonly found, and are not rare in the rabbit (Kohn[18]). Many isolated instances of their occurrence have been described in man (Jendrassik,[19] Amman,[20] Kohn,[18] Kürsteiner,[21] Harman,[16a] Erdheim[22] and others). Kürsteiner found that these accessory lobes have in some instances a diameter of 8 mm. or more, but might, on the other hand, be so small as to be visible only on microscopic section. Detached lobes of thymus having this origin have been found in the dog and in the rat (Kohn,[18] Pappenheimer,[23] Flesch[24]). Finally, in all animals in which the thymus migrates into the thorax but in which atrophy of the cervical strand is a normal occurrence, isolated lobes of thymus derived from the third pharyngeal pouch may be found at any point along its course.

As has been stated already, a thymus anlage from the fourth pharyngeal pouch, thymus IV, does not develop constantly in any of

17. Bien, Gertrud: Ueber accessorische Thymuslappen im Trigonum caroticum, Anat. Anz. **29**:325, 1906. Ueber accessorische Thymuslappen im Trigonum caroticum bei einem Embryo von 17 mm. grösster Länge, Anat. Anz. **31**:57, 1907.

18. Kohn, A.: Studien über die Schilddrüse, Arch. f. mikr. Anat. **44**: 366, 1894. Studien über die Schilddrüse, Arch. f. mikr. Anat. **44**:398, 1896.

19. Jendrassik, A. E.: Bau der Thymusdrüse, Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl., Wien. **22**:75, 1858.

20. Ammann, A.: Beiträge zur Anatomie der Thymusdrüse [Basel] Zürich, 1882, Orell, Füssli & Co.

21. Kürsteiner, W.: Epithelkörperchen und Thymusstrang beim Menschen, Cor.-Bl. f. schweiz. Aerzte **30**:638, 1900.

22. Erdheim, J.: Ueber Schilddrüsenplasie, Beitr. z. path. Anat. u z. allg. Path. **35**:366, 1904.

23. Pappenheimer, A. M.: Further Experiments on the Effects of Extirpation of the Thymus in Rats, with Special Reference to the Alleged Production of Rachitic Lesions, J. Exper. M. **20**:477, 1914.

24. Flesch, M.: Experimentelle Thymusstudien. I. Teil. Thymus und Milz bei der Ratte. Beitr. z. klin. Chir. **95**:376, 1915.

the higher mammals. In the majority of them it occurs only occasionally, and in one species, at least, is said not to occur at all. In the guinea-pig no thymus anlage develops at the fourth pharyngeal pouch and the parathyroid from the fourth pouch is frequently rudimentary or absent on one side (Ruben[14]). The thymus lobe derived from the fourth pouch is, on the other hand, exceedingly common in the cat, (Groschuff,[15] Kohn[16]) and is usually found with parathyroid IV completely surrounded in the substance of the thyroid, but may lie almost entirely outside it.[25] It is frequently present also in certain of the hoofed animals and has been reported in the rabbit (Groschuff and Kohn), the rat (Kohn, Klose, Pappenheimer and Flesch) and in human beings (Groschuff, Grosser and Betke, Klose, Yanase and others). With what frequency thymus IV develops in the dog and the rat is not perfectly clear. The rather vague statements of Klose would indicate that it was of frequent occurrence in both species, particularly the latter. Whether he actually found it in his experimental animals, however, or merely inferred that it must have been there, when his experiments turned out negatively, and no rests could be found elsewhere to explain that outcome, is not perfectly clear. Pappenheimer, who studied in serial section the tissues of the neck of his thymectomized rats (including the thyroids) for thymus rests, failed to find the lobe of thymus from the fourth pouch oftener than in one out of ten animals. When present in the different species, thymus IV is in the form of a small lobe often not much larger than a pinhead, but may be as large as the thyroid itself. In animals in which parathyroid IV becomes imbedded in the substance of the thyroid the thymus lobe is usually also imbedded, but it may remain in part or wholly outside the thyroid. It is usually identified by its situation in the substance of the thyroid and its intimate relationship to parathyroid IV. Histologically, it is exactly like the thymus derived from the third pouch. Its importance from the standpoint of experimental thymus extirpation, which has probably been greatly exaggerated, lies in the fact that, ordinarily at least, it cannot be recognized at operation, and there is no way by which it could be removed, even if its presence were suspected, short of removal of the thyroids themselves.

It is thus seen that the hoofed animals are poor subjects for complete thymectomy. Complete removal of the thymus can rarely, if ever, be accomplished in the cat because of the frequency with which

25. The accessory thymus lobe derived from the third pouch lying in contact with the thyroid is separated from the latter by the thyroid capsule, but the accessory lobe from the fourth pouch is intermingled more or less with the thyroid and cannot be removed completely without removing the thyroid also (Groschuff[15]).

accessory lobes of thymus occur in the substance of the thyroids and in the neck of that animal.[26] Moreover, the fascial investment of the thymus in the cat is so tough as to make the removal of the visible portions of the gland a matter of extreme difficulty. Although possessed of an entirely cervical thymus, the guinea-pig is also unsuitable for complete extirpation of the gland because of detached lobes of thymus close to the main body of the gland, but so minute and in such intimate relationship to the parathyroids from the third pouch and the thyroids as to make their separate removal impossible. Despite our lack of experience with the rabbit as an experimental subject we should suppose that it was impossible to remove the gland completely from that animal with any degree of regularity on account of the frequent occurence of accessory lobes about the thyroids. It has been proven by Pappenheimer that the thymus can be excised completely from the rat in the majority of animals. The dog is in all probability the best subject for complete thymectomy. At least, so far as our knowledge extends, isolated lobes of thymus inside and outside the thyroid are more infrequent in the dog than in any other of the species considered. The discoveries of accessory lobes of thymus in new-born infants by Grosser and Betke,[27] by Kürsteiner[28] and by Yanase[29] in a large proportion of infants and children examined makes us suspect, however, that accessory lobes of thymus may occur in the dog with a much greater frequency than has been thought hitherto and that, if special studies of the cervical tissues of new-born puppies were made with that end in view, such accessory lobes might be found in a large proportion of them also.

HISTORICAL REVIEW

Restelli[30] was the first of a long series of investigators to attempt to determine the nature of thymus function by removal of the gland. He performed experiments on ninety-eight animals (seventy-two sheep, twenty-three dogs, three calves), only six of which (four sheep, one calf and one dog) survived. It can be assumed from our knowledge

26. In a series of nine cats examined by one of the writers thymus lobes in the thyroids occurred in all, in seven of them in both lobes, in two of them in only one lobe. In all nine cats a lobe of thymus was found just outside the thyroid on at least one side.

27. Grosser, P., and Betke, R.: Epithelkörperchen-Untersuchungen mit besonderer Berücksichtigung der Tetania infantum, Ztschr. f. Kinderh. **1**: 458, 1910.

28. Kürsteiner, W.: Die Epithelkörperchen des Menschen in ihrer Beziehung zur Thyreoidea und Thymus, Anat. Hefte, Wiesb. **11**:391,1898.

29. Yanase, J.: Ueber Epithelkörperbefunde bei galvanischer Uebererregbarkeit der Kinder, Wien. klin. Wchnschr. **20**:1157, 1907. Also: Jahrb. f. Kinderh. **67**:57, 1908.

30. Restelli, D. A.: De thymo observationes anatomico-physiologico- pathologicae, Ticini Regii ex typog. Fusi et soci, 1845. 46 p. 4°.

of the distribution of the thymus in the hoofed animals and of the method employed by Restelli to remove it, that his extirpations in the sheep and the calf were grossly incomplete, and without much doubt were incomplete also in the dog. Moreover, all of his animals seem to have been infected (fever, emaciation, pus in the wound, empyema, pneumonia, etc.), and to have died in consequence in from nine to twenty-three days after the operation. The only surviving dog lived seventeen days. It is clear, therefore, that Restelli's experiments have only an historical interest, and that no inference in regard to the effects of removal of the thymus can be drawn from them.

Friedleben's[31] celebrated monograph on the thymus gland still remains the greatest single contribution to that subject. He removed the thymus from three goats and fifteen dogs. The extirpation in the goats was limited to the cervical thymus and was, therefore, partial. In the fifteen dogs the removal was thought to be complete in seven, and was known to be partial in five; in the remaining three animals extirpation of the thymus was combined with extirpation of the spleen. The age of the dogs at the time of operation ranged from 6 days to 4 months.

Friedleben made an incision one inch in length along the inner border of the left sternomastoid muscle at the base of the neck, tore through the fascia with his finger until the thymus was exposed — in so doing almost invariably producing a left-sided pneumothorax — caught the thymus with a pair of blunt forceps and drew it slowly and carefully out of the thorax. If he did not succeed in removing the gland *en masse* in this way, he attempted to take it out piecemeal.

The results of Friedleben's operations on the dog were as follows: Three dogs, considered completely thymectomized (Exps. 1, 2 and 4) died within two weeks after the operation of causes clearly unrelated to deprivation of thymus function. Two dogs (Exps. 5 and 11) operated on on the sixth and tenth days, respectively, developed normally, and showed negative necropsy findings when killed seventeen and forty-seven days later. One dog (Exp. 6), 2 months old at the time of the operation, remained in a healthy condition for twenty-two days and was then killed. At the necropsy the organs were found to be normal, except the long bones of the extremities, which showed "congestion," widening of the marrow cavity, thinning of the cortex and diminished rigidity. The last of the seven complete thymectomies (Exp. 12), Klose terms Friedleben's "single important experiment,"

31. Friedleben, A.: Die Physiologie der Thymusdrüse in Gesundheit und Krankheit vom Standpunkte experimenteller Forschung und klinischer Erfahrung. Ein Beitrag zur Lebensgeschichte. der Kindheit, Frankfurt a. M., literarische Anstalt, 1858, 336 p. 8°.

and laments that he did not appreciate its significance. In describing this experiment Klose[32] remarks: "This animal was entirely well until four months after the operation, then became emaciated, devoured cork and all sorts of stuff, was taken with attacks of colic and died in most extreme emaciation." Klose sees in Friedleben's account of the terminal condition of this animal the "stage of cachexia" which he himself described as a specific result of removal of the thymus, but does not allude to Friedleben's own explanation of the dog's illness and death, viz., that, passing out of his oversight, the animal received improper food, developed chronic diarrhea with attacks of colic and died in such an attack, and at necropsy showed masses of "taenia serrata" in the small intestine. "The death in this case was brought about entirely independently of the operation, through accidental infection of the alimentary tract" (Friedleben[31]).

Little need be said in regard to Friedleben's partial extirpation experiments either in dogs or goats. He left a small thymus rest in only one of the partially thymectomized dogs; in the other four dogs he left rests varying in size from two-thirds to probably one-half of the entire gland. All the partially thymectomized dogs developed normally and showed no lesions at necropsy. Friedleben noted in one of them, however, (Exp. 10), 4 months old at the time the partial thymectomy was performed and killed forty days afterward, that the thymus rest appeared swollen and succulent, as if hypertrophied, and weighed 11 gm. (180 "gran") as compared with 2.5 gm. (37 "gran"), the weight of the piece previously extirpated. Of the three partially thymectomized goats, one died of tetanus, the second died from an accident soon after the operation; and the third, which was 6 weeks old at the time of the operation, and allowed to live for eight months, showed normal conditions at necropsy.[33]

Friedleben regarded splenectomy as more dangerous than thymectomy, apparently on account of peritonitis, and thought that the removal of both thymus and spleen was necessarily fatal. All three subjects of the combined extirpations died, the first two (Exps. 19 and 20) almost immediately after the splenectomy, which was the second

32. "Der einzige Fall, der wichtig ist, aber von Friedleben aus äusseren Gründen schlecht beobachtet, darum leider gering bewertet und ungenügend untersucht wurde, ist der zwölfte. Dieses Tier war bis zu 4 Monate nach der Operation ganz gesund. Dann magerte es ab, frass Kork und alle möglichen Gegenstände, litt an kolikartigen Anfällen und ging in extremster Abmagerung ein." Klose, H.: Chirurgie der Thymusdrüse, Stuttg., 1912, F. Enke. 299 p. 8°. N. deutsche Chir., hrsg. von P. von Bruns, Bd. iii.

33. The thoracic thymus of this goat (only the cervical thymus was removed at operation) weighed less than the thoracic thymus of a normal, somewhat lighter control animal, but showed no signs of involution, in contrast to the thoracic thymus of the latter, which was somewhat involuted.

operation; the third (Exp. 18), operated upon when 6 weeks old, survived three and one half months but died, finally, in a condition of marasmus without any discoverable cause.

Friedleben also made metabolism studies and chemical analyses of the bones of his thymectomized animals, which need not be described here.

As the result of his investigations Friedleben reached the conclusion that the thymus gland was not essential to life, but that the thymus and spleen together were essential, and that the death which followed the removal of both these organs was one of wasting brought about by a failure in the process of blood formation caused by the cessation of their combined activities. Nevertheless, he regarded the thymus as an important organ whose functions were especially concerned with blood formation, nutrition and growth. In contrast to many of the investigators who came after him he concluded that animals deprived of the thymus grew faster than normal animals, though not in proportion to their appetites, which seemed to him excessive.[34]

When the operative method employed by Friedleben for removal of the thymus is considered, doubt is raised whether any of his thymectomies could have been complete, and, indeed, only one of the seven experiments in which he thought the thymus completely removed merits consideration, quite apart from the question whether the gland was completely removed or not. It is, therefore, impossible to draw any conclusions regarding the nature of thymus function from Friedleben's experiments. Yet imperfect as they are, they have had great influence in the field of thymus investigation, for they not only showed that extirpation of the thymus was possible, thereby pointing out a way of approach to the problem of thymus function, but suggested in considerable measure the lines along which subsequent investigations have moved. For example, his experiments directed Basch's attention to a possible relationship between thymus function and growth of bone, and the theory of a specific interrelationship between thymus and spleen, put forward into such prominence by Klose, is Friedleben's conception somewhat amplified. Friedleben's experiments Nos. 10 and 23 are regarded by some writers as having an especial importance and are often cited as the first experimental proofs that the thymus possesses an especial capacity to undergo regeneration.

Viewed after a lapse of almost fifty-nine years, it seems inconceivable that the publication of Friedleben's monograph, which brought together not only all the existing knowledge of the time with regard to the thymus but, far more important, the results of Friedleben's own fundamental investigations, should not have called forth immediate

34. He never observed perversion of appetite in any thymectomized animal.

attempts to learn the secret of thymus function by experiment. Yet all effort toward this end lay dormant for thirty-five years, and when Langerhans and Saveliew broke the spell by the publication in 1893, of the results of their experiments, it was without so much as an intimation of a knowledge on their part of the existence of Friedleben's work.

The experimental animals of Langerhans and Saveliew[35] consisted of twenty-nine rabbits, aged from 3 to 5 weeks, and two dogs. The method of operation employed by them was essentially the same as Friedleben's in the dog, namely, withdrawal of the thymus from the thorax by traction through an incision in the soft parts at the base of the neck. Three of the rabbits were excluded from consideration because the thymus was not completely removed; six died immediately as a result of the operation, and nine within the first twelve days and one sixty and another sixty-seven days afterward, apparently from gastrointestinal disorders. The authors state that the nine rabbits that remained were alive and in a normal condition at the date of completion of their paper, but at what age they leave to the imagination of their readers. (Some of the thymectomized rabbits grew to be at least two months old.) They thymectomized "completely" only one of their two dogs, which was alive and well ten weeks after operation. The results of their experiments were thus all of a negative character. Their only contribution to their subject of investigation was in method; they were the first to control the operative animals with others from the same litters.

In the following year Tarulli and Lo Monaco[36] made a preliminary report of the results of two thymus extirpation experiments on puppies (ages at the time of operation not stated), controlling the animals operated on from the same litters. They found that the blood of the thymectomized animals showed an increase in the number of eosinophils immediately after the operation and a few days afterward a diminution in the hemoglobin and in the number of red blood cells and a slight leukocytosis, all of which continued for one and one half months, but by the end of four months had disappeared entirely. The two dogs operated on developed voracious appetites soon after the operation, but at the same time put on weight less rapidly and appeared less vigorous than the two control animals. Moreover, the hair of the former did not grow so rapidly and became coarse, lost its lustre and could be pulled out easily.

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35. Langerhans, R., and Saveliew, N.: Beitrag zur Physiologie der Brustdrüse, Virchow's Arch. f. path. Anat. [etc.] **134**:344, 1893.

36. Tarulli, L., and Lo Monaco, D.: Sugli effetti della estirpazione del timo, Atti d. xi. Cong. med. internaz., Roma **2**: (fisiol.) 19, 1894.

Three years later (1897) Tarulli and Lo Monaco[37] reported *in extenso* a series of thymus extirpation experiments performed on both puppies and chickens. The operation used by them for removal of the thymus in the puppy merits a special description. An incision was made in the median line from a point about 4 cm. above the manubrial notch to a point from 3 to 4 cm. below it. The sternocleidomastoid and sternohyoid muscles were divided close to their points of origin from the thorax. The wound was retracted toward the left, so as to expose to view the base of the neck and the subclavian region on the left side. The dome of the left pleura was ruptured from above with forceps in order to produce a pneumothorax, and the costal cartilages of the first and second ribs were divided close to the sternum with the object of obtaining a direct exposure of the thymus within the thorax. The latter organ was then drawn out of the thorax slowly by means of forceps. (Apparently it usually was brought out piecemeal.) The wound was closed in the ordinary way. Tarulli and Lo Monaco were thus the first to enter the thorax boldly through an incision in its wall in pursuit of the thymus.[38]

The dogs which Tarulli and Lo Monaco used in their thymus extirpation experiments numbered sixteen, divided into six groups of one animal for operation and one control animal each, and one group of three animals for operation and one control animal, all members of each group from the same litter. One puppy was only 12 days old at the time of operation and was observed for almost four and one half months afterward. Another puppy was 14 days old at operation and was kept under observation for four months. Another puppy was only 19 days old when operated on, and was watched for almost five and one half months; and still another puppy, thymectomized when 28 days old, was observed for five months. The remaining puppies, from 20 to 90 days old when operated on, were observed for short periods of from fifteen days to one and one half months. Some of the animals died from causes which had no apparent relation to deprivation of thymus function; the others were killed or were dismissed from further observation while in normal health. Necropsies were performed on the majority of the thymectomized dogs, but with the idea of determining that the thymectomies were complete rather than to discover finer histologic changes in the viscera.

Tarulli and Lo Monaco studied their animals especially for (1) alterations in the blood; (2) alterations in growth and nutrition as measured in terms of weight; and (3) alterations in muscular strength

37. Tarulli, L., and Lo Monaco, D.: Ricerche sperimentali sul timo, Bull. d. r. Acad. med. di Roma **23**:311, 1897.

38. The next advance in the development of the operation for removal of the thymus was made by Basch.

as judged by a comparison of the weights that the animals operated on and the control animals could drag along the ground.[39] They found that a few days after operation there occurred a decrease in the number of red blood corpuscles and in the percentage of hemoglobin and a slight increase in the number of white blood cells, but that in from three to four months after operation conditions in the blood had again become normal. Similarly, a retardation in the rate of growth of the thymectomized animals occurred soon after operation, which continued for one and one half months or more and then ceased. For two or more months after the operation the thymectomized animals were found to be less strong than the control animals, but afterward they gradually recovered their strength until they became quite as vigorous as the control animals. Tarulli and Lo Monaco further observed that from ten to fifteen days after operation the hair of the dogs operated on was noticeably shorter, coarser, drier and looser than the hair of the control animals; moreover, this disturbance in the growth of the hair did not disappear as the animals grew older, unlike the changes just enumerated. In the case of one thymectomized dog (Dog B, Exp. 3), operated on on the nineteenth day of life, there was observed a loss of the normal pigment of the hair to such extent that the brown hair of the animal turned white. This animal also showed curvature of the legs[40] two months after operation, a condition which was still present three months afterward at the time of necropsy.[41]

The authors thought that the deprivation of thymus function caused a lowered resistance of the animal for the first few days after operation, inasmuch as several thymectomized animals died without showing adequate cause at necropsy.

For the description of the operation which Tarulli and Lo Monaco used to remove the thymus in the chicken the reader is referred to their original article. The following brief account of their experiments on chickens is sufficient.

They removed the thymus from both sides of the neck in eighteen chickens, from 2 to 8 days old. Fifteen of the chickens died within seven or eight days after the operation, showing weakness of the legs, staggering gait, fine tremor involving the entire body, anemia with increase in the number of white cells, and at the end stupor. Two of the eighteen chickens survived and were normal again twelve days after the operation. Six chickens were operated on on one side only. One of these died after two days, with the symptoms described above;

39. The weights were attached to the tails of the animals.

40. The legs of this dog were described as being slender.

41. Tarulli and Lo Monaco do not give any particulars in regard to the deformities of the legs and do not include any statement in regard to the pathologic findings in the bones of this animal in their necropsy report.

five survived, showing some weakness of the limbs soon after the operation and only then. Those chickens whose thymus glands were removed completely when they were 25 days old showed no symptoms of any sort.

Abelous and Billard[42] published the results of their experiments on frogs in 1896. They selected the frog, because it had hitherto been untried, was anatomically well adapted for thymectomy and possessed a thymus which was thought to persist and for that reason might be considered to exercise throughout life an undiminished function. No frogs, from which both lobes of thymus were removed, survived the operation longer than from three to fourteen days. From twenty-four to forty-eight hours after the operation the animals showed lassitude, muscular weakness which passed over into a paralysis, loss of normal color of the skin, ulcers, especially in the neighborhood of the points of operation, progressive anemia accompanied by leukocytosis, ecchymoses, edema and death. If only one lobe of the thymus was removed, the animal did not die, unless already in a debilitated state, and none of the severe symptoms developed. But when the lobe which had been left was removed in its turn, the symptoms just enumerated supervened, with fatal termination. After one lobe of the thymus had been removed, the other lobe showed definite hypertrophy at the end of twenty days. Transference of blood from an affected to a normal animal caused the development of the characteristic symptoms, and produced death in the latter. Transplantation of a small piece of thymus under the skin of a thymectomized frog, affected not too severely, caused the faded skin to brighten after one hour, even to the point of return of the original color, but did not prevent death. Similarly, injection of extract of calf's thymus restored color to the affected skin, provided the condition was not already too advanced, and caused the animal to pass from a state of muscular weakness to hyperexcitability.

Abelous and Billard drew the conclusion that in the frog the thymus is essential to life. They also advanced a theory of thymus function based on their experiments, viz., that the thymus elaborates some substance which neutralizes a toxin formed in the course of normal metabolism, so that, when thymus activity is abolished, the organism becomes overwhelmed by the toxin and the characteristic train of symptoms ending in death follows.

The results of the experiments of Abelous and Billard appeared particularly clear cut and decisive. Was it possible that the thymus of the frog, which persists throughout life, had a different, a more

42. Abelous, J. E., and Billard: Recherches sur les fonctions du thymus chez la grenouille, Arch. de physiol, norm. et path .8:898, 1896.

important or a more sustained function than the thymus of the higher mammals? These questions were soon answered.

Vincent[43] found that "In the case of frogs, extirpation of both thymus glands does not necessarily end fatally." Some of his thymectomized frogs survived for thirty-six days after the operation, at the end of which time they either died from causes independent of the operation or were killed.

Though Camia[44] confirmed Abelous and Billard's results, others failed to do so. Ver Eecke[45] found that the symptoms noted by Abelous and Billard in thymectomized frogs did not occur if the water in the frog tanks was changed frequently, but developed if the water was allowed to stagnate. The frogs recovered when removed to a medium of frequently changed, fresh water, provided the symptoms had not progressed too far. Normal frogs exposed with the thymectomized animals remained unaffected.

Accordingly, ver Eecke expressed the belief that the pathologic condition developed by his thymectomized frogs was due to an infection from contaminated water and not to destruction of the thymus, and suggested that Abelous and Billard's results might be explained in the same manner. He was not certain, however, that the discrepancy between his results and theirs might not be due to a difference in material, inasmuch as he had used fasting winter frogs, but Abelous and Billard had used summer frogs.

Hammar[46] removed the thymus on both sides, or else on one side only, from 100 frogs and retained sixteen, which he subjected to sham operations,[47] as control animals. Eighty-five of the experiments were successful. Hammar found that removal of one lobe or of both lobes of the thymus in the frog produced no effect, whether the operation was performed in the spring or fall, or whether the animals were fed or not. He found that the thymus rests in his incompletely thymectomized frogs did not show signs of hypertrophy, but in some instances,

43. Vincent, S.: On the Results of Extirpation of the Thymus Glands, Proc. Physiol. Soc. Lond. **30**:16, 1903.

44. Camia, M.: Ricerche sulla funzione del timo nelle rane, Riv. di patol. nerv. **5**:97, 1900.

45. Ver Eecke, A.: Contribution à l'anatomo-physiologie du thymus chez la grenouille. [Rap. de O. Vanderstricht.] Bull. Soc. de méd. de Grand **66**: 140, 1899. Also: Ann. Soc. de méd. de Gand **78**:103, 1899. Structure et modifications fonctionnelles du thymus de la grenouille. [Rap. de Van Bambeke, 27.] Bull. Acad. roy. de méd. de Belg. **13**:67, 1899.

46. Hammar, J. A.: Ist die Thymusdrüse beim Frosch ein lebenswichtiges Organ? Einige experimentelle Untersuchungen, Arch. f. d. ges. Physiol. **110**: 337, 1905. Fünfzig Jahre Thymusforschung. Kritische Uebersicht der normalen Morphologie, Ergebn. d. Anat. u. Entwcklngsgesch. **19**:1, 1910.

47. The same operative steps as those required for removal of the thymus but without removal of latter.

on the contrary, showed involutional atrophy. He sectioned serially the necks of all his frogs operated on, so that it is certain his extirpations were complete in those animals in which he stated they were complete.

Hammar concludes his article with this sentence: "Thymus ist offenbar beim Frosch ebensowenig wie bei Säugern ein lebenswichtiges Organ." "The thymus obviously is an organ no more essential to life in the frog than in the mammals."

Pari[48] completed the evidence against the truth of Abelous and Billard's work by isolating an organism from the blood and organs of thymectomized frogs that had developed during confinement in unchanged water the characteristic clinical picture described by Abelous and Billard, and succeeded, further, in producing the disease in both thymectomized and normal frogs by inoculation with the organism. He found that although about one-third of the completely thymectomized frogs succumbed to the infection, only three of a total of about sixty "incised" frogs and three normal frogs succumbed, and, further, that there was a tendency for the disease to manifest itself locally in the "incised" animals, but in the thymectomized animals the infection was generalized. Accordingly, although he recognized that the symptom complex described by Abelous and Billard was caused by an infection and not by deprivation of the thymus, he thought that loss of the thymus was not without its effect, rendering the animals more susceptible to the particular infection in question and probably to all infections. In other words, Pari, like some other investigators, considered that a relationship existed between thymus function and immunity.

The last of the series of investigators to study the relationship between thymus function and development in the frog was Adler.[49] He extirpated the thymus by means of the galvanocautery from 950 tadpoles, but met with an extremely high mortality, so that the number of surviving thymectomized animals fell to thirty-two within a week after the operations. By the end of three months only twenty animals survived. These were killed and examined. The thymus proved to be completely extirpated in only three frogs; in five it was found that only one lobe had been destroyed and in the remaining twelve frogs portions of both lobes were still present. None of the animals operated on

48. Pari, G. A.: Sulla diminuzione della resistenza alle infezioni, come causa della morte delle rane dopo l'estirpazione dei timi, Gazz. d. osp. **26**: 321, 1905. Sulla causa della morte delle rane private dei timi, Atti r. Ist Veneto di sc., lett. ed arti, Venezia **65**:799, 1905. Also transl., Abstr., Arch. ital. de biol. **46**:225, 1906.

49. Adler, L.: Metamorphosestudien an Batrachierlarven. I. Exstirpation endokriner Drüsen. B. Exstirpation des Thymus, Arch f. Entwcklngsmechn. d. Organ. **40**:1, 1914.

showed abnormal growth or development. The testicles of the three thymus-free animals were larger than the testicles of the control animals. The pineal glands and hypophyses cerebri of these three frogs showed no changes, but the thyroids exhibited hyperplasia with infoldings of the epithelium and diminished colloid. In spite of the actual paucity of successful experiments, Adler was not deterred from formulating the general conclusion that a specific relationship exists in the frog between the thymus and thyroid and between the thymus and testis.

Carbone,[50] whose work is available to us only in abstract, removed the thymus from rabbits and from a single dog. He could detect no abnormalities in the animals operated on, however, except a transitory diminution in the number of red blood cells. According to Matti the weights of Carbone's animals indicate that they were too old to be expected to react positively to loss of thymus.

Ghika[51] reported a series of thymus extirpations in cats and rabbits. The technic of the operation was the same in both species, consisting in the removal of the thymus from the thorax by means of traction with forceps and blunt dissection through an incision in the neck extending from the sternal notch to the thyroid. In the cat it was necessary, in addition, to dissect out the two cervical horns of the thymus.

Ghika operated on eight cats varying in age from 15 days to 1 year, but was aware at the time of operation of leaving behind pieces of thymus in all but one animal. The cat, in which Ghika considered extirpation complete, emaciated and died one month and four days after the operation. The partially thymectomized cats showed no constant changes.

Ghika's extirpation experiments on the rabbit numbered fourteen, but were for the most part unsuccessful, and need not be described.

Cozzolino[52] removed the thymus from twenty-four rabbits weighing between 170 and 665 gm. at the time of operation (ages of rabbits not stated). Seven rabbits died of pneumonia almost immediately after operation; one rabbit died soon afterward (cause of death not stated), and two rabbits succumbed to diarrhea in from fourteen to seventeen days afterward. The experimental animals of Cozzolino, which must be considered, number fourteen. In these fourteen animals Cozzolino could not detect any disturbance of appetite, change in the hair, alteration in growth or in the corpuscles of the blood (examina-

50. Carbone, T.: Esperienze sull' estirpazione della ghiandola timo, Gior. d. r. Accad. di med. di Torino **45**:561, 1897. Abstr. Centralbl. f. allg. Path. u. path. Anat. **9**:365, 1898. Also: Centralbl. f. Chir. **24**:1280, 1897.

51. Ghika, C.: Etude sur le thymus, Paris, 1901, G. Steinheil, 228 p. 8°.

52. Cozzolino, O.: Risultati della stimizzazione nei giovani conigli, Lavori d. Cong. di med. int. 1903, Padova **13**:230, 1904. Intorno agli effetti dell' estirpazione del timo nei giovani conigli, Pediatria **1**:144, 1903.

tions made of blood from three thymectomized and three control rabbits). The negative observations of Cozzolino on the effects of thymectomy are not of great value, however, for the following reasons: He kept his rabbits for comparatively short periods after operation, the longest of which was sixty-seven days and the average not more than one month. Moreover, he injected diphtheria toxin into six of the fourteen rabbits in order to gage the resistance of thymusless animals to the poison of an acute disease, thereby causing their illness and death and depriving them of a value, which, otherwise, they might have possessed for the study of the effects of thymectomy. Furthermore, though he controlled the thymectomized rabbits used for injection with diphtheria toxin and for blood examination, he failed to control the others. Finally, he used the inferior Friedleben method for the removal of the thymus. He states, however, that necropsies showed his extirpations to have been complete. He made no histologic examination of the organs.

Though Wiesel, Klose and Matti all remark that Cozzolino found his thymusless rabbits had less resistance to diphtheria toxin than the control animals, such was not actually the case. Cozzolino, himself, states specifically that he was unable to reach any definite conclusion in regard to that question, for two thymectomized rabbits injected within seven days after the operation died before their control mates, and three rabbits injected more than one month after operation outlived their control mates.

In a subsequent article Cozzolino[53] reported two more thymus extirpation experiments on rabbits with positive results. Neither ages nor weights at the time of operation are mentioned. Three months after operation, bowing of the forelegs with swelling of the articular ends of the bones was noted. Both rabbits lost weight rather rapidly and died, one eleven days, the other fifteen days, after the first changes in the legs became apparent. At necropsy no lesions in the internal organs were found. The bones examined showed a pathologic condition which Cozzolino regarded as signifying an increased proliferation of the cartilage. The cartilage not only appeared to be overabundant, but extended in an irregular manner into the shaft and was more highly vascularized than normal. He concluded from the two experiments that a relationship of some sort existed between thymus function and normal growth of bone and considered the possibility that the bone changes might have been rachitic.

Unfortunately, neither of the two rabbits had a control animal, so that it was impossible to exclude rickets of other than thymoprivic origin. Cozzolino states his intention of repeating his experiments

53. Cozzolino, O.: Deformazioni dello scheletro degli arti nei giovani conigli in seguito all' ablazione del timo, Pediatria **1**:620, 1903.

properly controlled. Whether he never did so or never again obtained positive results, we do not know. We cannot find reference to any further work of his on this subject in the literature.

Sinnhuber[54] removed the thymus through a median incision in the sternum from two dogs 6 weeks old. In one of the dogs he implanted the thymus in the peritoneal cavity immediately after its removal. At the end of five days, when the two dogs had recovered from the operation, he made calcium determinations on their excreta (urine and feces), collected during three three-day periods, and the excreta of a control animal from the same litter, without finding any evidence that deprivation of thymus function altered the calcium output. Three times he repeated the calcium determinations on the excreta similarly collected during three three-day periods without obtaining positive results, but he complicated the last two determinations by introducing thyroid and thymus feeding into the diets of the animals. The latest periods chosen for study of the calcium output were forty-seven days after the operation.

Sinnhuber did not make a necropsy on the two thymectomized animals of his experiments to determine either the absence of thymus rests or the fate of the transplants or to discover whether changes existed in the organs. Nevertheless, he drew the sweeping conclusions that the thymus is not essential in postfetal life, and that neither extirpation of the thymus nor thymus feeding produces any change in the calcium output, and that the thymus is not concerned in any way in the development of rickets.

Vincent[43] removed the thymus from a series of guinea-pigs aged from 10 days to 1 month, but was unable to detect any disturbance in growth or any change in the blood.

Paton and Goodall[55] performed thymus extirpation experiments on the guinea-pig. They found that removal of the thymus was followed by a decrease in the number of lymphocytes for a period of about two months without coincident alteration in the other elements of the blood, but they observed no disturbance in growth and no other variation from the normal.

In 1905, Paton[56] published further thymectomy experiments on guinea-pigs, in which special study was made of the effects produced on the genital organs. The animals of his experiments consisted of twenty-four guinea-pigs, from one day to one and one half months old at the time thymectomy was performed, and they were killed at varying

54. Sinnhuber, F.: Ueber die Beziehungen der Thymus zum Kalkstoffwechsel, Ztschr. f. klin. Med. **54**:38, 1904.

55. Paton, D. N., and Goodall, A.: Contribution to the Physiology of the Thymus, J. Physiol. **31**:49, 1904.

56. Paton, D. N.: The Relationship of the Thymus to the Sexual Organs, J. Physiol. **32**:28, 1905.

periods after operation. The control animals were selected from the same litters when possible, but when this was impossible (and he does not indicate how often this was) they were matched with the thymectomized animals according to weight. Inasmuch as he had no record of the dates of birth of his animals, he was compelled to estimate their ages from their weights. Since he found that sexual maturity in the guinea-pig occurs at about the third month, when the animal has reached a weight of approximately 300 gm., he assumed in his experiments that animals weighing less than 300 gm. were less than three months of age and sexually immature, but that animals weighing more than 300 gm. were more than three months of age and sexually mature. The first group of six guinea-pigs (there were six control animals) weighed between 75 and 145 gm. at the time of operation and were allowed to live from two to six weeks; they were killed at such times as to make the final weight of all members of the group between 100 and 200 gm. The average postmortem weight of the animals operated on was 165 gm., of the control animals, 152 gm.; the weight of the testes and epididymes of the animals operated on was 0.23 gm.; the weight of the testes and epididymes of the control animals was 0.18 gm.. The second group of sixteen animals (there were fourteen control animals) weighed from 84 to 280 gm. when the thymus was removed, and they were killed at the end of four weeks—in two instances at the end of eight weeks—when the weights of all animals ranged between 200 and 300 gm. The average final weight of the guinea-pigs of this group operated on slightly exceeded that of the control guinea-pigs, but the average weight of the testes and epididymes of the thymectomized animals was 0.88 gm., whereas that of the control animals was only 0.60 gm. The two groups just mentioned comprised the immature guinea-pigs of Paton's experiments. The third group, composed of only two animals operated on (there were three control animals) represented his entire stock of mature subjects. One of the two guinea-pigs weighing 250 gm. at the operation was killed at the end of three weeks when it weighed 310 gm. The other guinea-pig, weighing 280 gm. when operated on, was allowed to live only one week. The testes, with epididymes attached, of these two animals, weighed almost the same (1.24 gm.) as those of the three control animals (1.33 gm.) Paton was unable to show that the loss of the thymus affected in any way the time of conception of female guinea-pigs.

Though it seems questionable whether Paton was entitled to draw definite conclusions from the experiments just described—except, perhaps, such general ones as that removal of the thymus in the guinea-pig is compatible with life, or that it does not produce any very obvious changes—he drew the specific conclusion that the thymus inhibits the growth of the testes in immature but not in mature animals; since

Calzolari,[57] Henderson[58] and others had already established the fact that the thymus continues to persist uninvoluted in animals castrated before maturity, he put forward the theory that prior to the period of sexual maturity thymus and sex glands act antagonistically, each exerting an inhibitory influence on the other.

Bracci[59] removed the thymus gland from eleven "young" rabbits, with the object of determining the effect on calcium metabolism. He used the Basch method of operation and regarded the removal of the thymus as a simple procedure. Only one of the eleven rabbits died as a consequence of the operation.

The plan which Bracci followed in his experiments is not perfectly clear but seems to have been as follows: Eight of the thymectomized rabbits were allowed to live for varying periods after operation, the longest of which was two months. They were controlled with a single rabbit on which a sham operation was performed. The two remaining thymectomized rabbits, and in addition four normal rabbits were injected with the sterilized filtrate of the aqueous solution of the thymus glands of the rabbits previously operated on, and were killed in from three to eight days after injection. Calcium determinations of the urine and feces were made every third day on each animal, in the thymectomized group, before and after thymectomy; in the injected group before and after injection of the extract.

Bracci's estimations of the calcium output and also of the calcium content of the organs in the thymus containing, thymus free and thymus injected animals came out with such a nicety of agreement as to indicate with extraordinary clearness, if correct,[60] a relationship between thymus function and calcium metabolism. The thymectomized rabbits showed a calcium excretion in excess of that which occurred before the thymus was removed, but those of them injected with thymus extract showed a lowered calcium excretion; the four normal rabbits also showed lowered calcium output so soon as they received injections of thymus extract. In perfect agreement with the above, the calcium content of bone, brain and blood (method of determination not stated) was found to be less in the thymectomized and greater in the injected animals than in the normal animals.

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57. Calzolari, A.: Recherches expérimentales sur un rapport probable entre la fonction du thymus et celle des testicules, Arch. ital. de biol. **30**:71, 1898.

58. Henderson, J.: On the Relationship of the Thymus to the Sexual Organs. I. The Influence of Castration on the Thymus, J. Physiol. **31**:222, 1904.

59. Bracci, C.: Timo e ricambio del calcio, Riv. di clin. pediat. **3**:572, 1905.

60. We cannot help but question experiments in which effects, the opposite of those produced by thymectomy, are obtained by injection of thymus extract. Consult Fischl, R.: Experimentelle Untersuchungen zur Analyse der Thymusextraktwirkung, Jahrb. f. Kinderh., **29**:385, 1914. Also: Verhandl. d. Versamml. d. Gesellsch. f. Kinderh. deutsch. Naturf. u. Aerzte, 1913, **20**:66, 1914.

Bracci examined the thyroids, ovaries, testes, suprarenals, liver, spleen, kidneys and bones of his thymectomized rabbits without finding gross or microscopic changes. In the bone marrow he detected numbers of eosinophils, numerous mitotic forms and megalokaryocytes with distinct pseudopodia.

In 1905, Fischl[61] published the results of his extirpations of the thymus in goats, dogs and rabbits. He did not perform the operations himself but had them done for him by a veterinary surgeon at a veterinary institute.

Fischl selected the goat as a good subject for thymectomy on the supposition that the thymus was situated entirely in the neck, an erroneous conception which would have been impossible had he either studied the goat before beginning his experiments or read Friedleben's description of the operation for thymus extirpation in the goat. The operation in the first series of goats was, therefore, limited to removal of the cervical portion of the gland. When Fischl discovered in the course of a necropsy on a goat, which he supposed had been rendered thymus free, a thymus in the thorax "as large as a fist," he found it necessary to subject the other goats already operated on to a second operation for removal of the thymus situated in the thorax. From the goats operated on subsequent to his discovery, however, both neck and breast thymuses were extirpated at single sittings. Fischl's goats were from 3 to 4 weeks old at the time of operation. Five died of septic conditions and eight survived. In these eight goats Fischl was unable to observe anything abnormal. Some of them, showing no abnormality after an observation period of more than six months, and consequently discarded as experimental subjects for the determination of thymus function, were used for brain operations, and it so happened that two of the goats in that way came to necropsy. In these two goats no thymus rests could be found. Fischl implanted a breast thymus from a normal goat in the peritoneal cavity of another normal goat without the production of hyperthymatization. Fischl fractured the forelegs of two normal goats and of two goats which had been thymectomized four weeks previously. He did not succeed, however, in detecting any differences in the healing process in the two sets of animals.

On account of the difficulty experienced in the removal of the thoracic thymus from the goat Fischl next turned his attention to the dog. He selected for operation dogs from 1 to 2 months old, weighing from 1 to 1.5 kg. The operation consisted in median incision of the neck with removal, in some instances, of the upper third of the manu-

61. Fischl, R.: Experimentelle Beiträge zur Frage der Bedeutung der Thymusexstirpation bei jungen Thieren, Ztschr. f. exper. Path. u. Therap. **1**:388, 1905.

brium for purposes of better exposure. Fischl lost five dogs from complications of hemorrhage and infection, and two dogs soon after the operation from distemper, so that the number of dogs became reduced to six thymectomized and two control animals. In none of the dogs did he obtain any changes whatsoever following incision of the gland. He produced fractures in the forelegs of three dogs one month after extirpation of the thymus and found that they healed exactly like similar fractures in the control animals as indicated by roentgen-ray examinations made at eight day periods. He trephined the skull of one of the dogs operated on one month after the thymus had been removed and found that healing took place in a normal manner. He reached the conclusion that extirpation of the thymus in the dog produced none of the changes which had previously been described by other investigators.

Fischl thereupon investigated thymus function in the rabbit. For this purpose he employed forty rabbits from 3 to 6 weeks old, a number which soon became reduced through operative mishaps and disease to twenty-five. Some of the twenty-five served as control animals. The operation on the rabbit consisted in ablation of the thymus through a median incision at the base of the neck. In some experiments the upper part of the manubrium was cut away or the cartilages of the upper ribs were incised close to their junction with the sternum, in order to afford better exposure of the gland. None of Fischl's successful extirpations of the thymus in rabbits were productive of any result whatsoever, with one possible exception — that of a rabbit which developed a paralysis of the hind legs and emaciation when 8 months old not explained at necropsy. Fischl weighed a group of thymectomized and control rabbits at intervals during the first two months after operation without bringing to light differences in their relative weights. He fractured the legs of eight thymectomized rabbits from four to five weeks after excision of the thymus and obtained perfect healing. Fischl does not give any definite information as to the length of time his rabbits were kept under observation. Apparently only a few of them were killed and subjected to a necropsy, and these only for some special purpose, e. g., to obtain a fractured bone. Fischl's experiments on rabbits were thus of a negative character.

Fischl[62] next investigated the effect of thymus extirpation on the fowl, being attracted by the remarkable results of Tarulli and Lo Monaco, which had never been confirmed or disproved. The report of his experiments on the fowl was published separately in 1907.

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62. Fischl, R.: Ueber die Folgen der Thymusausschaltung bei jungen Hühnern, Verhandl. d. Versamml. d. Gesellsch. f. Kinderh. deutsch. Naturf. u. Aerzte **24**:417, 1907. Also: Monatschr. f. Kinderh. **5**:329, 1907.

It is necessary to say but little in regard to these last of Fischl's extirpation experiments. The chickens ranged in age from one to twelve days at the time of operation. The thymus was removed from both sides in twenty-four and from only one side in two chickens. Two chickens died very shortly after the operation from causes unrelated to loss of thymus function and the others thrived so soon as they had recovered from the immediate effects of the operation and had been allowed to run loose.

As the result of his negative experiments in four different species of animals Fischl concluded that removal of the thymus is without significance to the organism and denied that experimentation furnished any proof of thymus function. Nevertheless, he did not abandon the idea that the thymus was an organ of internal secretion but, strangely enough, postulated for it a function in fetal life which ceased at birth, after which time the thymus continued to exist, according to his belief, only in a morphologic sense.

Fischl's work has been vigorously assailed by Basch,[63] probably with justification. Chickens and goats are not well adapted for thymectomy experiments, as several writers have pointed out. We have no assurance that Fischl's extirpation of the thymus gland in the dog and rabbit were complete, especially since he did not go to the trouble to exclude the presence of even large rests of thymus by means of necropsy, except in a few animals. Finally, his presentation of his experimental work is so unsystematic and lacking in essential details as to make it difficult to believe that the experiments themselves could have been conducted in a careful and orderly manner.

Basch published the preliminary report of his experiments in thymus extirpation in 1902[64] and 1903,[64] and the report in extenso in 1906.[65] His paper, dealing particularly with the relationship between thymus and nervous system, appeared in 1908.[66]

Basch's experiments mark a distinct advance in the investigation of thymus function. The most notable contribution which he made, the importance of which will be disputed by no one, was that of an operative method which simplified the removal of the thymus and imparted to the results of thymus extirpation a validity which they

63. Basch, K.: Bemerkungen zu Rudolf Fischl's Experimentelle Beiträge zur Frage der Bedeutung der Thymusexstirpation bei jungen Thieren, Ztschr. f. exper. Path. u. Therap., **2**:195, 1905.

64. Basch, K.: Ueber Ausschaltung der Thymusdrüse, Verhandl. d. Gessellsch. deutsch. Naturf. u. Aerzte, 1902, Leipzig **2**: 2. Hlfte., p. 322, 1903. Also: Wien. klin. Wchnschr., **16**:893, 1903.

65. Basch, K.: Ueber die Ausschaltung der Thymusdrüse, Jahrb. f. Kinderh. **64**:285, 1906.

66. Basch, K.: Beiträge zur Physiologie und Pathologie der Thymus. II. Ueber die Beziehung der Thymus zum Nervensystem, Jahrb. f. Kinderh. **68**:668, 1908.

had not possessed previously. To those accepting Basch's work at its full value his experiments are monumental in the history of internal secretion, establishing a definite and important connection between thymus function and the development of bone and between the thymus and the nervous system, and elevating the thymus to a position among the most important and best understood organs of internal secretion. Even to those who do not accept Basch's work his experiments appear to have been excellent in conception and execution. Basch's earlier investigations were concerned chiefly with the effects of removal of the thymus on the skeleton; his later ones centered on the changes produced in the electrical conductivity of the peripheral nerves.

After preliminary experiments, which Basch states he made on a variety of animals, including rabbits, frogs, pigeons, guinea-pigs, cats and dogs, he selected the dog as the subject best adapted for his purpose and found that small dogs, such as the rat terrier (Rattler) and fox terrier (Pinscher) were the most suitable. He controlled from the same litters. He operated in the third or fourth weeks of life, selecting that period because of the desirability of removing the thymus at the earliest moment compatible with safety, while the gland was still at the height of its function. He killed most of his animals in the second month after extirpation, but kept several pairs under observation for a longer time, and one pair for about six months.

Basch discovered in his earlier experiments that it was impossible to remove the thymus completely by means of the old method through an incision in the soft parts at the base of the neck; therefore, he carried his incision from the base of the neck along the middle of the sternum as far as its lower third and divided the latter, thereby laying open the thorax and exposing the thymus to view. He removed the thymus by dissection from above downward, then closed the thorax and suturing the soft parts over it with all possible speed, made it airtight again. A pneumothorax always occurred on the left side, and sometimes on the right. Yet Basch states that experience made him so adept that he lost no animal from among the last five litters and, when death seemed to impend from asphyxia owing to collapsed lung, it was possible to hold the edges of the wound in contact and save the animal by artificial respiration.

Basch removed the thymus in this way from twenty litters of puppies and in seventeen succeeded in obtaining at least one operative and one control animal which survived the date of operation for the minimal period of one month. In animals from fourteen of the seventeen litters there occurred pathologic changes in the bones which were all of the same general character, though differing in degree,

and they were demonstrable clinically, by roentgen ray and on post-mortem examination. In the animals from three litters apparently no changes of any kind developed. Basch also studied the effects of thymectomy on growth (weight) in one litter of rabbits.

Two or three weeks after operation Basch observed differences in the bones of the extremities of thymectomized and control animals; the legs of the former seemed the softer and more flexible. This difference was first apparent in the hind legs, especially in the tibias, when the attempt was made to bend them. He noted also that the animals operated on had a more straddling, clumsy gait, that they were less active, often sitting on their haunches to rest, and that their hind quarters were thinner and their hind legs appeared to be rotated outward. In a short time he perceived that the forelegs were bowed outward and enlarged in the region of the lower radial epiphyses and that the animals themselves began to walk with the fore feet, from the ankle downward, flat against the ground. The thymectomized dogs dropped behind their control animals in growth, in both height and weight, and showed less intelligence, those most affected seeming stupid.

In order to obtain a more exact experimental proof of the relationship of growth of bone to thymus function, Basch produced fractures of the tibias coincidently in animals operated on and in control animals in order to compare the reparative processes. In most of his animals he fractured the bones by bending between the hands, but in some instances by means of a chisel introduced through an incision in the soft parts. If the fracture was performed at a proper interval after thymectomy, Basch noted that the bone of the thymectomized animal broke with less force than the bone of the control animal. For the first four to five days after the fracture had been produced both animals behaved in an identical manner, but on the fifth day, the thymectomized animal began to use the broken leg, while the control ran about still sparing his. This difference was most striking on the sixth and seventh days, but after the eighth day the control dog also began to use the fractured leg and the difference in gait gradually disappeared. On the fourth and fifth days after fracture a difference in the callus formation could be detected. The callus of the control animal was clearly larger and more spindle shaped; in the thymectomized animal it was smaller and more nearly ring shaped. The difference in the callus formation was often apparent for four weeks after the fracture had been made. In one pair of animals Basch trephined holes in the tibias and found by roentgen-ray examinations that closure of the hole occurred more rapidly in the control animal. In the case of two thymectomized dogs suffering from diarrhea bony union did not seem to take place at all, so that a false joint was formed.

The difference in the healing of the fractures in the two sets of animals was most marked when the thymectomy was performed in young puppies, still suckling, and the fractures made from three to four weeks after the date of operation. When the fracture was produced from ten to twenty days after thymus extirpation the differences were less; and when made several months after the extirpation they were scarcely distinguishable. If the thymus was not removed entirely, the difference in callus formation between control and thymectomized animals was not so evident. In one instance, in which removal of the thymus had been incomplete, Basch saw signs that the thymus rest had hypertrophied.

In two thymectomized dogs the changes in the long bones were so similar to a case of spontaneous rickets in a dog which had come under Basch's notice as to suggest to him that the changes in the thymectomized animals might have been rachitic in nature. He concluded, however, that they could not have been so because of their short duration and for the most part mild character.

It is important to note that the differences which Basch observed between the animals operated on and the control animals were transitory. He states specifically that in the thymectomized animals, allowed to live from five to six months, the changes in the skeleton, which have been described, no longer existed. We suppose from Basch's statements that the thymusless animals overtook their mates in growth.

Basch made examinations, both gross and microscopic, of the bones of the thymectomized animals and roentgen-ray studies of the healing fractures. He also made some metabolism studies.

Gross examination of the bones of thymectomized and control animals, Basch states, showed those of the former to be shorter and thinner, the pars compacta to be less uniform and the trabeculae more delicate. The pathologic differences which he described in the callus formations about the points of fracture in the two sets of animals are such as would be inferred from the clinical descriptions already given.

The description which Basch gives of the finer changes in the bones of the thymectomized dogs is so vague as to make a clear understanding impossible. According to his statements the bones of the thymectomized animals remained retarded in development, particularly in respect to calcification; the changes were most marked in the metaphysis; the cartilage seemed to persist longer and ossified bone to be correspondingly diminished in amount; compact bone was less abundant; the marrow was more spongy, the trabeculae finer and containing a great deal of cartilaginous substance; the epiphyseal line was broader and more wavy and the nuclei of ossification less advanced. In one thymectomized animal the intermediary zones of

cartilage of the lower epiphysis of the femur seem to have been lacking.

Basch made metabolism experiments on two pairs of thymectomized and control dogs to determine the effect of loss of thymus function on calcium excretion. His first experiment was performed on a pair of dogs 9 weeks old, from one of which the thymus had been removed three weeks previously. The tibias of both dogs had been fractured one week before the metabolism experiment was begun, and the differences in healing of the fractures in the two animals were at the time clearly demonstrable. Both animals were put on identical diets and the urine was collected over a period of eight days. The feces were discarded. Basch found that the urine of the thymectomized dog contained more than twice as much calcium as the urine of the control animal. In a second experiment Basch measured the calcium excretion in both urine and feces of a pair of larger dogs, one of which had been thymectomized five weeks previously, and both of whose hind legs had been fractured seven days before and at the time of the metabolism determinations showed characteristic differences in callus formation. In the eight day period the thymusless dog excreted a total of 0.297 gm. CaO, and the control dog a total of 0.106 gm. CaO. In a later six day period, which was instituted fourteen days after the end of the eight day period just mentioned, the thymusless animal excreted 0.288 gm. and the control 0.333 gm. CaO.

It is probably not justifiable to draw any conclusion from the first of Basch's metabolism experiments, in which the calcium output in the urine alone was considered. The results of the two determinations in the second pair of animals are contradictory. Basch attempts to bring them into agreement, however, by attributing the lower calcium output of the animal operated on in the second period to fecal retention.

Basch concluded that the thymus is not essential to life but exercises a transitory function corresponding to its own life history; that its function is concerned especially with the growth and development of bone and intimately connected with the process of calcification. He suggested at least that growth bears some relation to the size of the thymus, supporting the theory by necropsy findings in a litter of three animals which, having exhibited in life great differences in size, showed at the postmortem correspondingly great differences in the size of their thymus glands.

Basch[67] repeated the thymus extirpation experiments of Fischl on the fowl in order to determine why the results obtained by Fischl were negative. He had previously attempted to remove the thymus

67. Basch, K.: Zur Thymusexstirpation beim jungen Huhn, Monatschr. f. Kinderh., **7**:541, 1908.

gland from a pair of pigeons, but had not succeeded because the thymus and fat tissue in that bird were too intimately intermingled. Indeed, when the two pigeons were examined after death — which occurred six and eight weeks after operation, respectively — Basch found so much new thymus tissue at the site formerly occupied by the gland that a continuous strand had been formed simulating the normal condition. He removed the thymus from a brood of chickens a week or less old and observed them for more than three months. At necropsy they showed exactly the same condition as that found in the pigeons. Thymus rests were in evidence at the normal site of the thymus in the form of long masses of tissue resembling in gross appearances lymphoid tissue. Basch detected no changes in either species of bird following thymectomy, and concluded not only that it was impossible to remove the thymus completely in the pigeon and fowl, but that it had an unusual capacity for regeneration. Basch credited Fischl's negative findings after thymectomy in the fowl to grossly incomplete extirpations.[68]

We shall not describe at length the part of Basch's[66] work in which he sought to establish a relationship between thymus and nervous system. He found that in thymectomized puppies there developed a hyperexcitability of the peripheral nerves (he used the median nerve in his experiments) to the galvanic current, after a proper latent period had lapsed. The difference in the strength of the current required to provoke contraction in the thymectomized and control animals amounted to three or four milliamperes. At the same time he was able to demonstrate in the thymectomized animals a hyperexcitability of the motor area of the brain, which was diminished by subcutaneous injection or local application of calcium chlorid and also by injections of thymus extract, which latter caused increased irritability in the normal animal. On the strength of these findings Basch interpreted the convulsions, which were common in his thymectomized dogs about three weeks after operation and were the cause of death in about one-tenth of them, as due to tetany.

From the results of these experiments Basch put forward the theory that not alone the parathyroids, but also the thymus was intimately concerned with the etiology of tetany, and that the thymus functionally as well as developmentally belonged to the branchiogenetic family of organs of internal secretion all with kindred or closely interrelated activities, and that in childhood the thymus stood in the foreground among them.

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68. Fischl has never replied to Basch's criticisms. Probably he could not do so, for there is no evidence in the report of his work on chickens that he ever looked to see whether thymus rests were present or not.

The roentgenograms and photographs of the bones of thymectomized and control dogs which form a part of Basch's papers are of importance, since they afford the reader the opportunity of drawing his own conclusions concerning Basch's work.

When the roentgenograms of the fractured bones taken at different periods during the course of healing are examined, it is seen that with two exceptions,[69] the fractures are of a more severe character in the control animals than in those deprived of thymus, i. e., the line of fracture in the former is the more distinct, the wider or the more irregular, or the displacement is the greater. A mere glance at Basch's roentgenograms will confirm this statement. In his series Nos. 25 to 32, for example, the point of fracture in the tibia of the thymectomized animal is scarcely visible, whereas the tibia of the control animal is marked by a broad, irregular break. If the fractures were actually more severe in the control than in the thymectomized animals, as the roentgenograms undoubtedly indicate, what more natural than that the callus formation about the fractures should appear larger in the former than in the latter! Indeed, if Basch's roentgenograms are illustrative of one thing more than another, it is that callus formation is greatest where injury to the bone is greatest. Yet Basch saw in these roentgenograms proof that the bone of the thymectomized animal had lost some of its osteogenetic energy.[70]

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69. One of the two exceptions in which the fracture in the bone of the control animal is not of a more severe character than the fracture in the bone of the thymectomized animal is shown in roentgenograms Nos. 33 to 38. The fractures seem to be of about equal severity, and it is noteworthy that the callus formations about the fractures are approximately equal in both animals, as would naturally be expected. Basch finds it necessary to explain the absence of the difference in the callus formations in the two animals, and does so on the ground that the fracture in the thymectomized animal was produced too soon after the thymus had been removed (ten days) for the osteogenetic capacity of the bones to be affected. The other exception is to be seen in roentgenograms Nos. 1, 2, 3 and 4. The roentgenograms of the fractured bones of the control are so overexposed that a comparison of the fractures in the two animals is difficult. Callus formation is, however, considerably greater about the fracture in the control animal.

70. If the injury to the bone produced by the fracture was less severe in the thymusless animal than in the control animal in Basch's experiments, restitution to the normal condition should have occurred more rapidly in the thymectomized animal than in the control, provided the reparative power of the bones in the two animals was equal. When Basch's series of roentgenograms of fractured bones are examined to determine in which set of dogs healing is further advanced, it becomes evident that in all but the two instances referred to in the previous footnote, healing is further advanced in the thymectomized than in the control animal, exactly as anticipated; in fact, in some of the roentgenograms and also in several of the photographs of longitudinal sections of the bone, the reparative process appears to be virtually complete in the bone of the thymectomized animal, while well marked callus formation with still visible line of fracture is present in the bone of the control animal. If, then, as Basch apparently assumes, the fractures in the bones of thymectomized and

Fig. 2.—Reproduced from Basch's article (Jahrb. f. Kinderh. **64**:285, 1906 [Fig. 45]), published by Basch to show the characteristic posture of the thymectomized animal after the symptoms have become fully developed. The dog is obviously sick and stands in a posture which indicates almost certainly confinement in a cage. There is nothing in the appearance of the animal to suggest rickets or any specific condition.

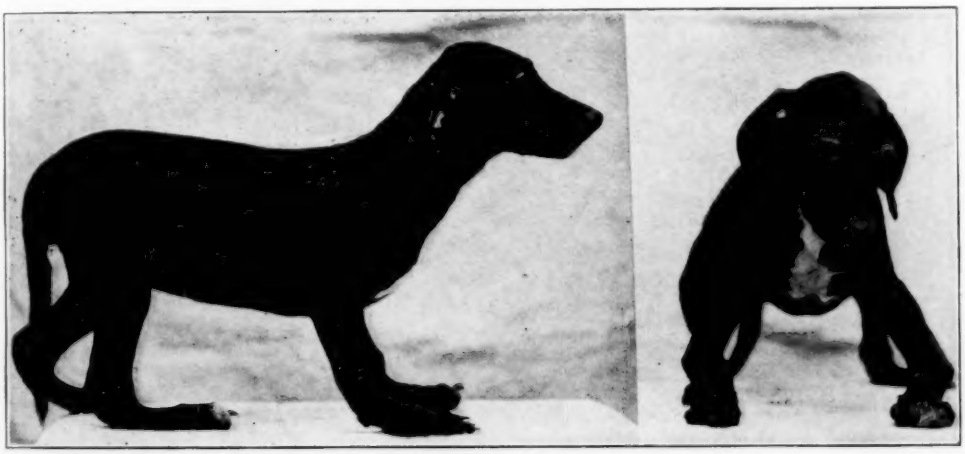

Fig. 3.—Side and front views of a dog, 5 months old, fed on a mixture of meat and corn meal and confined in a cage from the sixth week of life. At necropsy the thymus weighed 2.8 gm. Note that the animal stands in the characteristic flatfooted posture emphasized by Basch as a typical development in animals deprived of the thymus. Note also that he exhibits all the deformities described by Matti and Klose as characteristic consequences of the loss of the thymus gland. Yet the dog was never subjected to an operation. His thymus was never touched. Three other puppies of the same litter, similarly fed and confined, developed the same changes. Two of them, though, remaining healthy, lost the power to stand. One control animal not confined remained normal.

Fig. 4.—Internal circulation of the thymus gland.

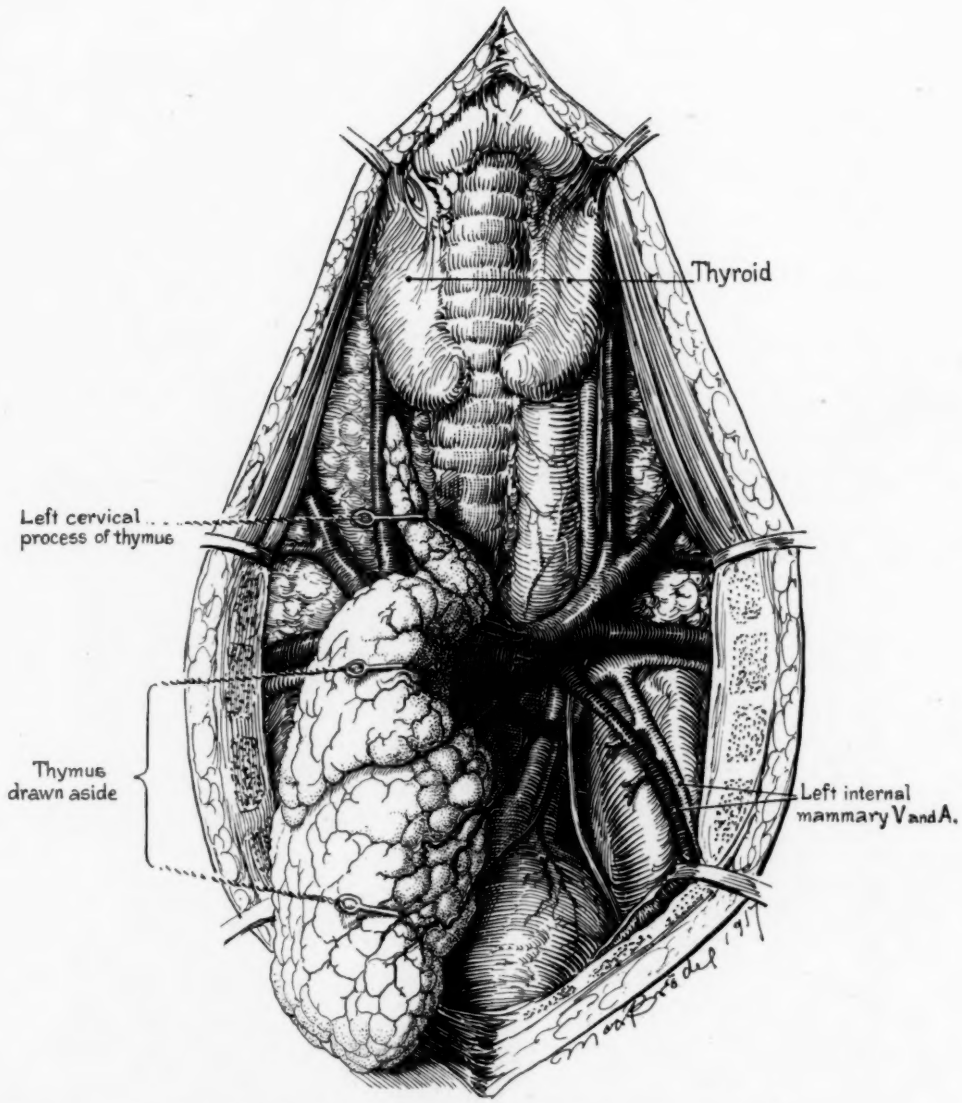

Fig. 5.—Showing the blood supply of the thymus gland.

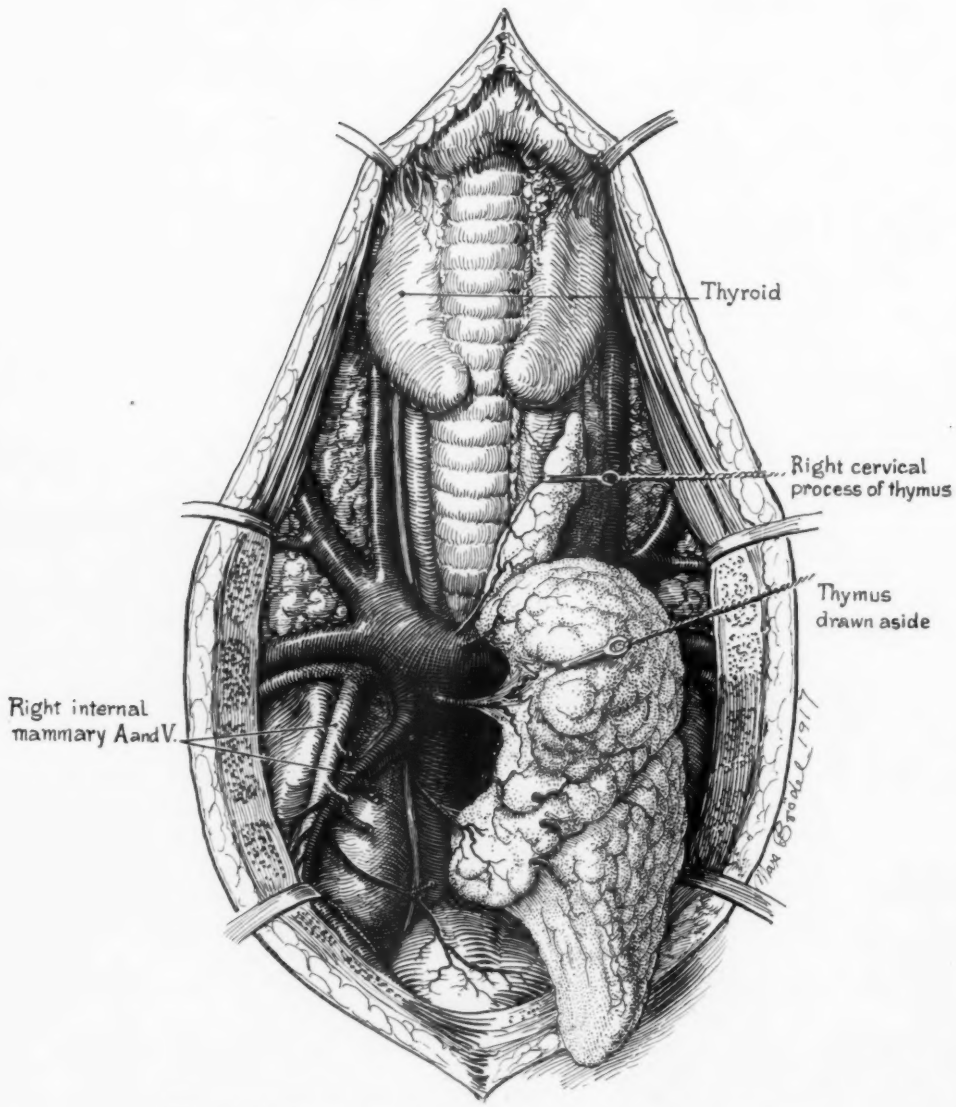

Fig. 6.--Showing the blood supply of the thymus gland.

The photographs of the longitudinal sections of the bones of the two sets of dogs are no more convincing of the truth of Basch's contentions than the roentgenograms are. In some of them callus formation is greater in the control, in others in the thymectomized animals. Incidentally, it should be noted that the bones of the thymectomized animals, exhibited for the purpose of demonstrating the character of the healing process after fracture, do not, with a single exception, show the retarded development of the centers of ossification. Indeed, in the roentgenograms the centers of ossification are the better developed in the thymectomized animals in the majority of instances. So, also, the fractured bones of the animals deprived of thymus appear to be, on the average, fully as long or longer than the bones of the control animals, though from Basch's description of the marked retarding influence of thymectomy on growth, we might have expected to find them shorter even in spite of the fact that the larger puppies were usually chosen for operation.

The bones showing the greatest disproportion in size in Basch's series of plates belonged not to thymectomized and control animals, but to a trio of normal puppies from the same litter with intact thymus glands, shown in support of the idea that the size of the animal was determined or influenced by the size of the thymus gland.

Basch's three photographs of living animals show two of the three thymectomized dogs to be greatly dwarfed and distinctly abnormal in appearance in contrast to their control animals. The third thymectomized dog, shown alone, in order to demonstrate "the characteristic position of fore and hind legs in sitting" has exactly the posture of dogs confined in cages and bears so striking a resemblance to a normal dog confined in a cage by us in the hope that he would develop rickets that we have published the photographs of the two animals (Basch's and ours) side by side (Figs. 2 and 3). One of the three low magnification drawings of bones of thymectomized animals (No. 60), (from one of the two animals in which Basch suspected rickets) is highly suggestive of rickets. The other two drawings (Nos. 58 and 59) indicate no more than osteoporosis. The single higher power drawing of a preparation from the bone of a thymectomized dog (No. 64) presents an absolutely normal appearance, except for an abrupt transition from less mature to more mature cartilage cells, a condition which is not found in normal bone, or in any disease and must be considered a unique finding, devoid of general significance.

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control animals were of equal severity in the first place, it would be necessary to conclude that deprivation of thymus function actually had stimulated the osteogenetic power of the bone and promoted more rapid healing, a conclusion which is directly opposite to the one drawn by Basch.

Not only do Basch's objective proofs of the effects of thymectomy in large part fail entirely, but the more careful reading of his clinical and pathologic descriptions leads to the impression that the changes which he describes in his thymectomized animals were not very definite. For instance, the "straddling gait" on which Basch laid such emphasis is not so significant in an animal whose sternum recently had been split and whose pectoral and cervical muscles had been divided or injured. The observation that the animal deprived of his thymus used his fractured leg two or three days earlier than the control can scarcely be interpreted as meaning that the thymectomized dog was pathologic![71] If it indicated anything it would be that the control dog was pathologic. If we eliminate from Basch's description those findings based on subjective impressions, there is really little left besides transitory retardation of development.

Whatever the pathologic condition in the bones of Basch's thymectomized dogs may have been, his description of the finer changes cannot be interpreted as more than osteoporosis.[72] Osteoid, apparently, was lacking.

It is disappointing that Basch has not given more information in regard to the manner in which his experiments were conducted, how his animals were kept, whether confined or allowed to exercise, what they were fed, and that more accurate descriptions and illustrations of the finer pathologic changes in the bone were not furnished. For it seems entirely possible to us that all the changes which Basch reported, both clinical and pathologic, might readily have been the result of confinement alone or confinement in combination with poor feeding, or disease. Indeed, Basch's observation that the pathologic condition in the bones of his animals deprived of the thymus was almost entirely limited to the extremities (the ribs being scarcely affected) is highly suggestive that he was dealing only with the results of "domestication" and not with some general disturbance in bone metabolism dependent on deprivation of thymus function, which undoubtedly would have affected the middle tier of ribs with especial severity on account of their extremely rapid growth, as is the case in rickets. Moreover, Basch reports an experiment which makes it seem still more probable that the pathologic condition in the bones of his thymectomized animals was in no wise dependent on deprivation

71. The precocious use of the fractured leg by the thymectomized animal is quite in keeping with our observation made from Basch's roentgenograms that the fractures were the more severe in the control animals, and Basch's other observation that the callus in the control animal was larger, more spindle shaped and persisted longer than the callus in the thymectomized animal, is open to the same explanation.

72. It seems not improbable, however, that the two thymectomized dogs whose bones showed a pathologic condition so different from that found in the other thymectomized animals as to necessitate a separate description actually had rickets, although Basch himself did not think so.

of thymus function. He states that he was able to bring about the same changes in the bone as followed removal of the thymus by destroying the gland with injections of paraffin into its substance. When it is considered that the thymus is a friable, ramifying organ, frequently sending processes into the pocket behind the left innominate vein and filaments along the great vessels in the neck, etc. (Plate 1, see frontispiece), and derives its blood supply from all the smaller vessels given off in the neighborhood, it becomes evident that it cannot possibly be destroyed or nearly destroyed in that manner (Figs. 4, 5 and 6). In this experiment, therefore, Basch must have obtained changes identical with those following removal of the thymus gland without destroying it and therefore without destroying thymus function.[73]

The criticism of Basch's work just given must not be taken to mean that we regard his findings as imaginary. Its purpose is to point out that his results are not nearly so convincing on careful examination as they seem to be on casual reading, and that a large part of his objective proof fails. If deprivation of thymus function causes transitory retardation in growth and pathologic condition in the bones of the extremities in the nature even of an osteoporosis, Basch has done a great service in bringing them to light. His work, up to the present time, has been accepted by many writers at its face value.

Soli[74] published a preliminary report of thymus extirpation experiments in cocks and rabbits in 1906, and in 1909[75] and 1910[76] more important papers dealing with the effects of thymectomy, especially on the growth and development of the skeleton and of the testes in cocks, rabbits and guinea-pigs and on the shell of the egg in pullets.[77]

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73. In another experiment, Basch states that he was able to prevent the occurrence of changes in the bones of thymectomized animals by transplanting the thymus gland to the abdominal cavity. Although a number of similar experiments with similar results have been reported by others, we are skeptical in regard to them and doubt the potency of a cause, the effects of which can be removed by such a procedure.

74. Soli, U.: Comportamento dei testicoli negli animali stimizzati, Policlin., sezz. med. **13**:529, 1906. Also transl.: Arch. ital. de biol. **47**:115, 1907.

75. Soli, U.: Contributo alla funzione del timo nel pollo ed in alcuni mammiferi, Path. Riv. quindicin., Genova **1**:149, 1908. Also transl.: Arch. ital. de biol. **52**:353, 1909. Sulle modificazioni dello sviluppo delle ossa negli animali stimizzati; nota preliminaire, Path. Riv. quindicin., Genova **1**:283, 1908.

76. Soli, U.: Modifications du développement des os chez les animaux privés de thymus, Arch. ital. de biol. **52**:217, 1909.

77. Soli published in both Italian and French, and though the more extended reports of his work are found in Italian, for each of them, with one exception, there is a corresponding report in French. His most important publication[78] unfortunately, is not available to us. It probably contains important details which are entirely lacking in the corresponding publication in French.

78. Soli, U.: Influenza del timo sul ricambio del calcio nei polli adulti, Path. Riv. quindicin., Genova **3**:118, 1910.

Soli[76] removed the thymus from two rabbits, the first 1 month old, the second 2 months old, controlling from the same litters. Both animals weighed less at the time of operation than did the control animals. One of them was killed after an observation period of one and one half months, the other after an observation period of three months. At necropsy both thymectomized animals again weighed less than their control animals and the volume and length of the bones were less. In the rabbit, which was the younger at the time of operation, two fractures were discovered in the fifth and sixth ribs and some minor abnormalities of a not very definite character were detected in the tibias. The bones of the other thymectomized rabbit showed nothing abnormal.

Soli also removed the thymus from eleven guinea-pigs (one adult pregnant female and ten young pigs from 1 to 5 days old), but found no alterations in the skeletons worthy of mention.

Soli seems to have attached great importance to the results of the two thymectomy experiments in rabbits and laid especial emphasis on the fact that the rabbit younger at the time of operation showed changes in the skeleton, whereas the older animal showed none. He writes: "Although the experiments are still few in number, it is proven that extirpation of the thymus has no influence on the skeleton, if the development of the latter has progressed to a certain point. If, on the contrary, the operation is practiced on younger rabbits, and if they are sacrificed after a short interval, alterations in the skeleton can be recognized. The results of these experiments are a new confirmation of the idea already put forward by a number of writers, and with a great abundance of arguments by Basch, of the functional importance of the thymus at a very early age."[79]

In regard to the effects on the skeleton of removal of the thymus in the guinea-pig Soli came to a different conclusion. "It seems to me, consequently, necessary to conclude that in the case of the guinea-pig extirpation of the thymus, even though it is performed in the first days of life, does not produce any noteworthy alteration in the skeleton."[80]

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79. Il est donc prouvé, bien que les recherches soient encore en petit nombre, que si le développmement squelettique a ateint un certain degré, il n'est pas influencé par l'extirpation du thymus. Si, au contraire, cette operátion est pratiquée chez des lapins plus jeunes, et si ceux-ci sont sacrifiés après une courte période de temps, on constate des altérations dans le développement squelettique. Ce résultat est une nouvelle confirmation du concept dé jà soutenu par plusieurs auteurs, et, avec une grande abondance d'arguments, egalement par Basch, de l'importance fonctionelle du thymus dans le premier âge, Arch. ital. de biol. **52**:221, 1909.

80. Il me semble par conséquent pouvoir conclure que, chez les cobayes, l'extirpation du thymus, alors même qu'elle est exécutée dans les premiers jours de vie, ne produit pas de notables altérations des os, Arch. ital. de biol. **52**:223, 1909.

Later in the same year Soli[75] published the results of his investigation of the effects of castration on the growth and involution of the thymus and on the other organs of internal secretion in rabbits and fowls, and, conversely, of the effects of removal of the thymus on the sexual organs and on some of the other endocrine glands in rabbits, fowls and guinea-pigs. We shall discuss only the experiments which have to do with removal of the thymus, as they alone fall within the scope of this paper.

Soli extirpated the thymus from one each of seventeen pairs of cocks, fifteen pairs of guinea-pigs and two pairs of rabbits, the other member of each pair serving as a control subject. He makes the statement that the control animals were of the same age and breeds as the animals operated on, from which we would infer that they were not all, at least, of the same broods or litters. Soli gives neither the ages nor the weights of his animals at the time when the thymus was removed, nor the length of time they were kept under observation, and though he records the weights of the testes of operated and control animals in parallel columns and the ratio of their weights to each other, he omits altogether the body weights of the animals from which the testicles were taken.

Soli states that removal of the thymus caused a delayed development of the testes in all three species of animals, which was most marked in the cock, less so in the rabbit and only slightly in the guinea-pig. In the fifteen pairs of cocks, the weights of whose testes are tabulated, the testes were heavier in the operated birds in all but two instances. Indeed, the differences in weight of the testes of animals operated on and control animals were so marked in some instances as to make it difficult to believe that the figures given could have been correct. For example, in one pair (7) the testes of the operated cock weighed only one forty-ninth as much as the testes of the control, and in another pair (10) the testes of the bird operated on were about one-thirtieth of the weight of the testes of the control bird. In two pairs of cocks the testes of the birds operated on actually weighed more than those of their control birds. Soli explained away the discordant results in the case of these two pairs on the ground that they, in contradistinction to the others, were thymectomized in the fall and killed in the spring, and goes on to make the following remarkable statement: "It seems to me then permissible to think that the connection between thymus and testes undergoes a modification with the approach of the period of rut, a period when, as has been observed in a large number of animals, it seems as if the testicle frees itself from all dependence of the rest of the organism and behaves like an

autonomous organ."[81] On the other hand, the differences in the weights of the testes in the guinea-pigs, as given in the tables, were so slight as to be negligible, although Soli did not so regard them. In nine pairs of thymectomized and control guinea-pigs the testes were practically equal in weight; in thirteen pairs they were heavier in the operated animals; in the remaining three pairs they were heavier in the control animals. In one experiment on the rabbit the testes of the thymectomized animal weighed five times as much as the testes of the control animal, in the other experiment twice as much. He obtained analogous effects by means of thymectomy on the ovaries of rabbits and guinea-pigs, which were much less marked in the latter than in the former.

Soli could not detect any disturbance in growth or development following thymectomy in any of the three species of animals or any definite changes in the spleen, thyroid, hypophysis or suprarenals. He concluded that extirpation of the thymus in young cocks, rabbits and guinea-pigs retarded the normal development of the testicle and ovary.

In 1910 Soli extended his thymectomy experiments to pullets in order to determine the effect of deprivation of the thymus on the deposition of calcium in the shell of the egg. His experiments performed with this object in view were so remarkable that they are presented in detail.

EXPERIMENT 1.—A pullet, without control, 1 year old, not confined, laid eggs regularly on alternate days. July 3 the thymus was removed. Between July 5 and July 15 she laid six eggs with normal shells; July 17, two weeks after the thymectomy, she laid an egg without a shell; July 19 she laid an egg with a shell; but between July 19 and July 30 she laid five eggs without shells. July 29 she was fed a combination of calcium carbonate, magnesium carbonate and calcium phosphate. Two days later, July 31, she laid an egg with an extremely thin shell; August 2 she laid an egg with a normal shell, and from that time on she has continued to lay eggs with normal shells. Two years after the operation she was killed and necropsied, but no trace of thymus was found.

EXPERIMENT 2.—Two pullets over 1 year of age; one bird was reserved as control. In May one pullet was thymectomized. During the sixteen days following the operation she laid seven normal eggs, but on the eighteenth day she laid an egg with an extremely thin shell, which was followed by two eggs with similar shells, then by two eggs devoid of shells and then by eggs with imperfect shells. Forty days after the operation her eggs became normal again. The control pullet, under exactly similar conditions and receiving the same food, laid eggs with normal shells throughout.

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81. Il me semble donc permis de penser que le rapport entre le thymus et les testicules subit une modification à l'approche de l'époque des amours, époque ou, comme on l'a, observé chez un grand nombre d'animaux il semble que le testicule se débarrasse de toute dépendance du reste de l'organisme et devienne comme un organe apparemment autonome, Arch. ital. de biol. **52**:367, 1909.

EXPERIMENT 3.—A pullet, age not stated, uncontrolled. For twenty days after thymectomy she laid normal eggs, then an egg with a thin shell, and from the twenty-third to the forty-fifth day she laid ten eggs entirely devoid of shell. From the forty-fifth day on she laid normal eggs.

EXPERIMENT 4.—A pullet, age not given, uncontrolled, laid on the eighteenth day after operation an egg devoid of shell, and during the following twenty days she laid nine eggs without shell; but beginning the thirty-ninth day after operation she again laid eggs with normal shells.

Soli performed a sham operation, leaving the thymus intact, in one pullet, and in another extirpated the "lower lobes" of the thymus, leaving the rest of the gland in situ, but the eggs laid by the two birds remained normal.

Soli concluded that calcium metabolism was modified in the hen by extirpation of the thymus and ascribed the failure in the calcification of the egg to diminished absorption of the calcium from the intestinal tract and to diminished utilization of the calcium circulating in the blood by the various tissues of the body.

The experiments of Soli just described are too few in number and too imperfect to permit of any final conclusion to be drawn from them. In only one experiment was the pullet operated on controlled, and in only one was the absence of thymus determined at necropsy.[82] Moreover, we lack sufficient information concerning the food of the thymectomized birds, especially in regard to its calcium content, to be certain that it could not have been an etiologic factor. Nevertheless, the results of Soli's experiments are so striking that, if they represent the truth, they are by far the most beautiful demonstrations of the dependence of normal calcium metabolism on thymus function to be found in the literature. Though it is highly important that these experiments should be confirmed or disproved, they have never been repeated by Soli or any other investigator, in so far as we are aware.

Soli's experiments on mammals are of far less importance. The experiments on the effect of thymectomy on the testicle in the guinea-pig were obviously negative, although Soli regarded them as positive; those on the rabbit were too few and imperfect to be of value; those on the cock cannot be accepted in the form in which they were presented by Soli without confirmation.

MacLennan[83] removed the thymus from rabbits 6 weeks old (number of experiments not stated). He made a median incision from a point just above the sternum caudalward for a distance of one inch, and cleared the sternum of muscular attachments. He then put ligatures of silk around each costal cartilage close to its junction with the sternum, leaving the ends of the ligatures long, and removed the sternum in its entirety by cutting through the costal cartilages mesial

82. Basch states that the thymus cannot be extirpated completely from the fowl.

83. MacLennan, A.: An Experimental Investigation Into the Function of the Thymus Gland, Glasgow M. J. **70**:97, 1908.

to the points of ligature. He next dissected out the thymus, and closed the thorax by drawing together the cut ends of the costal cartilages, uniting them to each other by tying together the loose ends of the ligatures. He then sewed together the soft parts.

Within a few days after operation the thymectomized rabbits became more vigorous and voracious than their brothers and sisters and showed greater resistance to infection. For example, when both sets of animals were attacked by favus, the control animals suffered most and the latter were more frequently affected with coccidiosis, enteritis and tuberculosis. The thymectomized rabbits increased more rapidly in weight than the control rabbits. Four animals, two control and two thymectomized animals, developed signs of rickets.

When the animals were killed (MacLennan does not indicate how long his animals were under observation), the spleens and the bone marrow were found to be normal. The cartilage of the epiphysis, however, appeared thickened. In MacLennan's words, there was present in the animals operated on a "greater number of steps between cartilage cell and bone cell" and "evidence of greater activity of growth." The thyroids of the thymusless animals were smaller and paler and on microscopic examination proved to be more cellular and to have smaller alveolar spaces and larger adenoid masses than those of the control animals.

MacLennan also performed thymectomy experiments on kittens. He does not describe any of them but merely states that the operation in the kitten is very severe because the heart is "almost embedded in the thymus, and it is nearly impossible to get the entire gland removed in every case." He characterizes the results obtained as "beneficial."

MacLennan's experiments are interesting from several standpoints. In the first place his method of operation for removal of the thymus is unique.[84] In the second place, the positive results which he obtained by removal of the thymus are, in the main, directly the opposite of the positive results obtained by all other investigators.[85] It is interesting to find MacLennan, on the one hand, prepared to advocate thymectomy as a therapeutic procedure and Klose, on the other hand, cautioning his readers against complete removal of the thymus in human beings lest it result in death. In the third place, MacLennan was the first to describe changes in the thyroid following thymectomy which have since been brought into such prominence by Matti.

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84. It has since been employed by Renton and Robertson.

85. His thymectomized animals grew faster, were more vigorous and more resistant to infections than the control animals; in short the removal of the thymus was, in MacLennan's words, "beneficial," while Klose, Matti and others found dwarfing, loss of energy, diminished resistance to infection and ultimately death. Friedleben was also of the opinion that deprivation of the thymus caused more rapid growth.

Sommer and Flörcken[86] performed thymectomy and thymus transplantation experiments on puppies and kittens 14 days old. If there were three in a litter, one animal was used for thymectomy, one for transplantation and the third as a control animal; if only two animals were in a litter, the transplantation experiment was omitted. Apparently, extirpations were actually performed on two cats and two dogs, and transplantations on two dogs. Their operation in both species of animals consisted in the removal of the thymus through an incision in the base of the neck without cutting the thorax. When a transplantation as well as thymectomy was contemplated, both subjects were put on the operating table together and the thymus of one was transferred immediately to a pocket made in the abdominal wall of the other.

Two months after the operation, Sommer and Flörcken observed striking differences between the thymectomized animals, on the one hand, and the control animals and transplant carriers, on the other. The former were distinguished by an awkward behavior, a clumsy gait and roughness and dryness of the hair. Their nutrition was fairly good. Roentgen-ray examination of the skeleton showed nothing abnormal. When the animals were 6 months old they were killed. No rests were found in the thymectomized animals, and no traces of the transplants were visible in the two animals used for transplantation experiments. The long bones of the extremities of the thymectomized animals at this time, however, showed definite changes. They were shorter and thicker than the bones of the other animals and could be bent relatively easily. The authors state that the calcium content of the bones was reduced, though they do not seem to have made either chemical or histologic examinations. The bones of the animals in which transplantations of the thymus had been made (the animals "with the two thymuses"), on the other hand, were longer and more slender than the bones of the control animals.

Sommer and Flörcken concluded from their few experiments that the thymus was not essential to life in the dog or cat but that it stimulated the growth of bone in postfetal life.

Ranzi and Tandler[87] presented a report of thymus extirpation experiments performed chiefly on dogs but also on sheep and guinea-pigs before the Association of Physicians of Vienna in 1909. So far as the experiments in the sheep and guinea-pig are concerned, the authors simply record the fact of their performance. They do not give even an intimation of the results obtained other than is contained in

86. Sommer, A., and Flörcken, H.: Ueber die Funktion der Thymus, Sitzungsb. d. phys.-med. Gesellsch. zu Würzb., 1908, p. 45.

87. Ranzi and Tandler, J.: Ueber Thymusexstirpation, Wien. klin. Wchnschr. **22**:980, 1909.

the passing remark that they were unable to confirm the results of the experiments of Paton as to the stimulating effect of thymus extirpation on the growth of the testicle in the guinea-pig. The operation on the dog, and probably also that on the sheep, was performed with the aid of the Sauerbruch chamber and the thorax was entered through a median incision in the sternum.

Ranzi and Tandler announced that removal of the thymus in the dog caused transitory retardation in growth and changes in the skeleton of varying degree, and in support of their statements demonstrated a litter composed of one dog, thymectomized three and one half months previously, when 6 weeks old, and a control animal. The thymectomized animal was the smaller and weighed less than his mate, exhibited the "straddling" gait described by Basch and still carried milk teeth in contrast to the control animal. In proof of the ephemeral character of the symptoms produced by thymectomy, they stated that in their experiment of longest duration the changes in the thymectomized animal had already undergone a retrogression.

In addition to the experiments just mentioned Ranzi and Tandler remarked that they had transplanted the thymus without effect, injected thymus extract into sheep, causing retarded growth, and tested the effect of thymus extract on the enucleated eye of the frog with contradictory results.

Except for the general statement of results and the few meager glimpses afforded in their brief report we are in complete ignorance regarding their work. They have never published anything further concerning the results of their experiments, so far as we can discover. They were the first to use a method for the maintenance of artificial respiration after the thorax had been opened.

We now come to the work of Klose whose extirpations of the thymus, performed on no less than five distinct species, were probably more than twice as numerous as those of any other operator on the gland in mammals,[88] and whose writings on the subject from the experimental standpoint have equalled or exceeded in volume the combined output of predecessors and contemporaries. It is scarcely necessary to add that Klose's influence in turning the course of scientific opinion in regard to the nature of thymus function has been considerable.

Klose's two earliest publications[3] on the thymus[7] appeared in 1910. The second was Klose's most important work. Published in conjunction with Vogt,[3] it contains a more extended report of Klose's important experiments, those performed on dogs, than any of

88. Klose does not give the number of his experiments. We know that he performed more than fifty-four successful experiments in the dog alone.

his subsequent publications.[89] His later writings,[91] with the exception of the most recent of them, which deals with the effects of thymectomy on the rat, goat and pig,[116] are very largely restatements of the Klose-Vogt publication. Klose's article, entitled "Chirurgie der Thymusdrüse,"[92] deserves special mention because it contains a very considerable modification of his former views on the results of thymus extirpation, due to the influence of Matti.

If Klose's earlier work can be said to consist essentially of a corroboration and amplification of the ideas of Basch, as has been intimated by Matti,[4] his later work may be regarded as a remodeling of his former experimental results and ideas so as to bring them in conformity with those of Matti.

His experiments on dogs performed with Vogt will be discussed immediately; his work of 1914, including the results of his experiments on the rat, goat and swine, will be considered after the presentation of the work of Matti.

Klose and Vogt used for their experiments ten litters of puppies, the two largest of eight, the two smallest of five puppies each, making a total of sixty-four. They removed the thymus from fifty-four, retaining but a single puppy in each litter as a control animal. They refer to their dogs as if they had been of thoroughbred stock—"terriers, poodles, etc."—but the photographs indicate mongrel stock, presumably with a preponderance of one breed or another. The breeds found to be best adapted for their experiments were terriers and poodles. The majority of the dogs were operated on either on the tenth or fourteenth day of life, but the dogs of one litter were not operated on until they were 3 weeks old and the dogs of still another litter were not operated on until they were 4 weeks old.

The operation used by Klose and Vogt does not require special description; it was the method of Basch with the modification that the thymus was dissected out of the thorax from below upward instead of in the reverse direction. In their joint experiments, Klose and Vogt did not employ any device for the maintenance of artificial respiration, but in subsequent experiments Klose used the "Vollhard-Meltzer"

89. This article, though published by Klose and Vogt, is a collaboration of several investigators including Liesegang, Schultz and Embden. Another investigator whose work was done in collaboration with Klose but whose articles were published independently was Lampe.[90]

90. Lampé, A. E.: Die biologische Bedeutung der Thymusdrüse auf Grund neuerer Experimentalstudien, Med. Klin. **8**:1117, 1912. Also: Abstr. München. med. Wchnschr. **59**:1833, 1912. The Function of the Thymus Gland, Am. J. Obst. **67**:808, 1913.

91. One of them[92] is the 283-page volume in the Neue Deutsche Chirurgie series devoted to the thymus.

92. Klose, H.: Chirurgie der Thymusdrüse, Ergebn. d. Chir. u. Orthop. **8**:274, 1914.

appliance for intratracheal insufflation. Twenty-five of the fifty-four thymectomized animals Klose and Vogt kept under observation until they died, "in order to obtain a perspective," remaining, "perfectly passive" while the pathologic changes developed to the fatal termination. The remaining twenty-nine thymectomized animals we suppose formed the material used for their subsidiary experiments.

If the operation was performed between the tenth and fourteenth days of life Klose and Vogt found that the development of symptoms occurred as follows: For at least fourteen days, and in many instances for two or three months after operation no change was noted. The first fourteen days after operation Klose and Vogt arbitrarily called the "latent period." But some time in the course of the two or three months following the operation, the observer who was "experienced" noticed characteristic changes. Although the weight curves of both thymectomized and control dogs ran essentially parallel, yet the soft tissues of the thymectomized dogs felt less firm and the dogs themselves appeared less vigorous and energetic; often they seemed obliged to sit on the haunches to rest. If the rigidity of the leg bones was tested, it was found that those of the thymectomized animals bent more easily than the corresponding bones of the control animals. The thymectomized animals became increasingly sluggish and at the same time began to manifest voracious appetites, often devouring twice the portions of their mates in spite of their lesser activity. Moreover, the appetites of the thymectomized dogs took on an abnormal character, in the sense that they gnawed sticks, stones, etc., as if they could not distinguish them from food. Changes in intelligence became evident. The thymectomized animals no longer seemed to recognize their keeper as formerly, or to know when the feeding hour fell due. They ate as if all sense of satiety had vanished. Their "gaze" at this period Klose and Vogt described as "dreamy and melancholy," often directed at a single point, as if the animals were in a trance. This period in the thymectomized dogs' illnesses, following the "latent period" and characterized by the symptoms just described, Klose and Vogt termed "the stage of adiposity." It was from two to three months long. If a necropsy was done during this period, the body was found to contain abnormally large amounts of fat and alterations in the bones were present, the exact nature of which Klose and Vogt do not disclose. At the end of the "period of adiposity," from two and one half to six months after operation,[93] a fundamental change was observable in the thymectomized animals. The weight curve of the latter, which previously had been ascending, suddenly began to decline, in spite of the fact that their voracious appetites had not abated. Weakness began to

93. Klose and Vogt are not perfectly clear in regard to the time division between the "latent" and "adipose" periods.

manifest itself, particularly in the bones of the legs and in the muscles. Moreover, the innate sense of self-protection of the thymusless dogs seemed to have become blunted, for they frequently injured themselves by falling or bumping into objects without seeming to be conscious of doing so. Often they sat on their haunches for minutes at a time with the forelegs spread wide apart apparently in the effort to maintain equilibrium. Occasionally, muscular tremors, coarser than those of tetany, became manifest. The expression of the thymusless dogs became entirely devoid of intelligence and two of the dogs mutilated themselves, as if they had lost all sense of pain. Finally, they seemed to lose even the sense of hunger, although their voracious appetites continued almost to the end. They developed at this time spontaneous fractures and became subject to all manner of infections, such as distemper, pneumonia, diarrhea, ulcerations of the cornea, and so forth, which in many instances were the immediate causes of death. Their hair became dry and fell out. Finally, unable to move about, for "the skeleton no longer was strong enough to support the body," and apparently devoid of all sense,[94] the thymectomized animals passed into a terminal coma and died.[95] The authors regarded the coma as a specific effect of loss of thymus and named it "coma thymicum." The period of illness just described, beginning with the end of the "stage of adiposity" and terminating in death, Klose and Vogt called the "stage of cachexia." The duration of this last stage varied from three to fourteen months. In animals, therefore, from which the thymus was removed in the period between the tenth and the fourteenth days of life, death inevitably occurred from three and one half to seventeen and one half months afterward.

But if the removal of the thymus was not accomplished until the third or the fourth week Klose and Vogt found the "latent period" much prolonged, the "stage of adiposity" requiring five to six months for full development and the "stage of cachexia" often extending from the seventh month into the beginning of the third year. Thus, though death was as inevitable after thymectomy in the third or fourth weeks, as after thymectomy between the tenth and fourteenth days, it might be deferred for a period of more than two years. If the thymus was not removed until the fifth or sixth week, either no symptoms were produced or else symptoms of a purely transitory character. *The third to the fourth week, then, Klose and Vogt found was the latest period in which thymectomy could be done and produce the characteristic changes above enumerated and then only in case breeds of dogs especially susceptible were used, as for example, poodles and terriers, and*

94. Klose and Vogt[3] remark, "The body of the animal is at the last a great ruin" (p. 77).

95. The death of the thymectomized animals Klose and Vogt refer to as "thymic death."

provided the operator had perfect technical skill. After the fourth week of life, Klose and Vogt state, complete removal of the thymus in the dog is anatomically and surgically impossible, and that thymectomies done on animals older than four weeks are not and cannot be regarded as valid experiments for the determination of thymus function.

As already indicated, Klose and Vogt laid the greatest emphasis on the evidence of involvement of the nervous system in the thymectomized dogs. The movements of the dogs appeared to them as "infantile"; the facial expression, toward the end of the "stage of cachexia," as "senile." The inability of the thymusless animals to find their kennels, their failure to show signs of recognition of their keeper, their uncleanly habits (defecation in their kennels), the insatiability of their appetites, their predilection for gnawing sticks and stones, the stupidity of their facial expressions convinced Klose and Vogt that the thymusless dogs had become idiotic! Accordingly, they named the disturbed state of intelligence of the thymectomized animals, developing during the "stage of cachexia," "thymic idiocy." "Thymic idiocy," like "thymic coma," they regarded as a specific condition produced only by deprivation of thymus function and an inevitable consequence of loss of the gland in the first four weeks of life.

Of all the pathologic changes which Klose and Vogt discovered following extirpation of the thymus, those in the skeleton were the most striking. It is to be regretted that in their large series of animals they did not trace the development of the changes in the skeleton from the beginning, for it would be most important to know not only the nature of the initial alterations but also the time of their occurrence with relation to the development of the various symptoms.

The well developed lesions in the bones of the thymectomized animals which alone Klose and Vogt described are essentially the following: The long bones of the thymectomized dogs were shorter than those of the control animals; for example, the femur of a thymectomized dog measured 104 mm., whereas the femur of the control animal measured 132 mm.[96] The flat bones of the skull were also smaller than those of the control animals.

Ossification in the thymectomized animals was not so far advanced as ossification in the control animals; for example, the first rib in the thymectomized animals was either completely cartilaginous and broad without a trace of perichondral ossification or else the cortex was extraordinarily thin and the spongy portion formed a wide, open

96. Klose and Vogt's smallest litters numbered five, and their largest, eight dogs. It is not clear why they selected only one of the thymectomized animals of the litter to compare with the control animal. We take it that the differences between the control animal and the other thymectomized animals were less marked or did not exist, or Klose would have given the measurements of the other operated animals also.

mesh-work, while the corresponding ribs of the control dogs showed complete ossification. So, too, the centers of ossification in the sternum of the thymectomized dogs were separate while those in the control animals had fused completely, etc. The rigidity of the bones of the thymectomized animals was judged to be, or to have been, less than that in the control animals. The sternal part of the thorax in the thymectomized animals was in many instances found to form a pigeon breast, from which fact Klose and Vogt inferred it had become so softened as to have been distorted by the diaphragm. So, too, the ventral surface of the thorax at the level of the shoulders was broader in the thymectomized animals, an observation which they explained on the ground that the thorax had been weak enough to yield to the divergent pull exerted through the fore legs. In contrast to the sternum and ribs, however, the bones of the extremities appeared to be brittle, for, on the one hand, they showed signs of the multiple fractures, which had been rendered visible during life by means of the roentgen ray, and, on the other hand, showed no evidences of bending at points not the site of fracture. The vertebrae, ribs and sternum of the operated animals cut readily with the shears, but those of the control animals cut only with the saw. When the bones of the thymectomized dogs were sectioned longitudinally, it was found that numerous cysts were present, particularly at the sites of the fractures, and these apparently were the causes of the fractures. Some of the cysts were of considerable size, as large as hazelnuts, whereas others were so small as to be visible only under the microscope. The epiphyseal cartilage was unequal in breadth and stained more faintly blue than normal. The cortex was thin and the marrow fatty. The articular cartilage was unequally thick. On microscopic examination, the epiphyseal cartilage appeared to be homogeneous but vacuolated and thicker than normal. Cells of the peripheral columns were larger than those of the central columns and their columnar arrangement was less clearly defined. The preparatory zone of calcification was smaller and more shallow. Osteoblasts were flatter and less numerous. The trabeculae were more delicate. Periosteal bone seemed to have also a diminished osteoblastic complement. *Finally, Klose and Vogt observed that the border of osteoid was not increased but actually diminished in width.*

Klose and Vogt considered that the pathologic condition in the bones of the thymectomized animals as revealed by the microscope could not be more than osteoporosis.[97] Chemical examination showed, how-

97. "On consideraion of all these microscopic findings there is no other diagnosis which can be made than atrophy." "Es ist auf alle diese mikroskopischen Befunde hin zunächst keine andere Diagnose zu stellen, als die der Atrophie."[3]

ever, that the calcium content was only from 32 to 34 per cent., while in the control dogs it was 65 per cent. of the total ash; in other words, the calcium content of the bone in the thymectomized dog was greatly reduced. This diminution in calcium caused Klose and Vogt to express the view that the condition in the bones of the thymectomized dogs might be related to rachitis or to osteomalacia. They finally settled their difficulty by concluding that it must be a combination of all three conditions.

Klose and Vogt also found anatomic changes in the nervous system, which, though relatively slight, afforded some sort of anatomic basis for their clinical observations. The peripheral nerves showed slight degrees of neuritis; the spinal cord slight degenerative changes in the gray substance with proliferation of glia; the brain as a whole was of definitely increased volume and edematous. The histologic changes in the gray matter were the most marked, consisting of swelling and edema of the ganglion cells and early degenerative changes in their nuclei with increase in the glia. The endothelial cells of the blood vessels were swollen and there was slight perivascular infiltration.

Klose and Vogt repeated the experiments of Basch on the healing of artificially produced fractures. They produced fractures of both fore legs and hind legs in six thymectomized dogs, but at a time much later than that selected by Basch, viz., in the "stage of cachexia," which, as will be remembered, began at its earliest in the third month and considerably later in animals thymectomized after the second week. At the time when they produced the fractures they observed that the bones of the thymusless animals broke with less force than those of the control animals and that it was impossible to fracture the bones with the free hand exactly at the point desired, for the break occurred wherever the bones happened to be weakest, which was at the site of the cysts, the location of which naturally could not be determined beforehand. Apparently in five of the six thymectomized dogs the bones were fractured between the hands of the operator, and in the remaining one by means of a chisel through an incision in the soft parts. The fractures in the control dogs, however, appear in all cases to have been produced by means of the chisel. Under these circumstances it would seem that the healing processes in the two groups of animals were scarcely comparable. Nevertheless, Klose and Vogt laid great weight on the differences found. They observed that the thymectomized dogs used their broken legs on the sixth day, whereas the normal animals used their legs on the tenth day. The roentgen ray showed on the fourteenth day after the fracture traces of bone formation in the control dogs' legs but scarcely so much as a circular callus in the legs of the thymectomized dogs. Three weeks after the fractures had been produced the bones of the control animals had firmly united,

but those of the thymectomized dogs still showed false points of motion. The callus formations in the thymectomized dogs, as seen with the roentgen ray, were by this time invariably larger than those of the control dogs. Definite spindle shaped callus formations were first visible by means of the roentgen ray in the healthy animals five weeks after the operation, while the fractures in the control animals, in spite of roentgen-ray appearances[98] exhibited the same imperfect union as two weeks after the fracture had been produced.

Klose and Vogt also repeated on some of their animals[99] Basch's experiments on the conductivity of the peripheral nerves to the galvanic current, as they state, confirming his findings. Their results were not, however, altogether uniform. Contradictory though it seems, they were able to produce the same electric changes as those obtained in animals deprived of thymus in two normal dogs by means of the injection of thymus extract and nucleic acid. They remark that their thymectomized dogs, unlike those of Basch, were not subject to convulsions. It will be remembered that the latter author regarded the convulsions as a direct consequence of loss of thymus.

Klose and Vogt found that no changes occurred in the blood of the animals after thymectomy except an anemia of a secondary character in the period of "cachexia"; that there was no temperature variation, constant or distinctive, and no constant variation in the nitrogen excretion as shown by determinations on a thymectomized and a control dog over a considerable period.

Among the more important and interesting findings of Klose and Vogt are those bearing on the relation of the thymus to other organs of internal secretion. These two investigators noted that following thymectomy there occurred an hypertrophy of the spleen, thyroid, pancreas and genital organs, as was shown by a comparison of their weights in some of the thymectomized and control animals of equal body weight. The table, giving only average figures, indicates that the spleens and thyroids of the thymectomized and control animals (number not stated), weighed almost the same, whereas the pancreas and genital organs of the thymectomized animals weighed almost twice as much as the corresponding organs of the control animals. Klose and Vogt state that microscopic examination showed that the pancreas, genital organs and thyroids of the thymectomized dogs presented no

98. Klose and Vogt pointed out that roentgenograms of healing fractures considered alone gave misleading impressions because union of the fragments by connective tissue callus, which would not cast much shadow, might have been sufficient to support the weight of the emaciated body of the thymectomized animal, whereas firm union was required to sustain the weight of the control animal.

99. No information in regard to number is given.

variation from the normal, so that their increased size must have been in the nature of simple hyperplasia. The spleen, however, exhibited constant pathologic changes, on which Klose and Vogt placed the greatest emphasis. These consisted in an increase in the number and size of the malpighian bodies, from three to five of which were frequently found in a single field, and in the presence of many giant cells in the pulp. These changes, however, were characteristic only in those animals killed during the "stage of adiposity." Indeed, quite the opposite condition obtained in the "stage of cachexia"; the malpighian bodies were then found to be almost absent and the pulp to contain abundant small granules, nuclear fragments and pigment masses, which they interpreted as evidences of destruction of the blood and of the fixed elements of the spleen. Klose and Vogt saw in the changes in the spleen occurring during the "stage of adiposity" the signs of hyperfunction, but interpreted those in the "stage of cachexia" as meaning that hyperfunction had given way to paralysis of all function.

Klose and Vogt also extirpated, in turn, several of the organs of internal secretion from dogs already thymectomized in order to determine the effects of removal of an organ of internal secretion in addition to the thymus. They found that if the thyroid glands were excised in addition to the thymus (the thyroids were removed from four thymectomized animals in the "stage of cachexia") not one of the animals lived longer than fourteen days, and concluded that removal of the thyroid shortened the "cachectic stage" and intensified the pathologic process induced by removal of the thymus without altering its character. They found evidence, also, of the closest relationship between pancreas and thymus, in that diabetes in its severest form supervened and produced death in twenty-four hours, when the pancreas was removed from dogs previously thymectomized (four experiments). They could not demonstrate a definite relationship between the thymus and the genital organs, for no changes of either quantitative or qualitative nature followed castration of thymectomized dogs during the "stage of adiposity" (four experiments). Extirpation of the spleen from thymectomized animals, however, proved to be entirely confirmatory of the conjecture which they had already made from their histologic studies.

Although, as has already been pointed out, late removal of the thymus, i. e., in the third or fourth week, produced death after a long period, from one to two years or more, and removal of the thymus in the fifth and sixth weeks produced transitory symptoms or no symptoms at all, it was found that removal of the spleen from an animal which had been thymectomized in the fourth, fifth or sixth week of life, caused death very rapidly, within a few weeks, and with the characteristic picture of "thymic cachexia." Moreover, if the spleen

was extirpated from an animal thymectomized early, that is, between the tenth and fourteenth days, typical thymic cachexia developed with the most extreme rapidity, terminating fatally within eight days or three weeks. Thus removal of the spleen appeared many times over to accelerate and intensify the pathologic processes set in motion by removal of the thymus.

As a result of experimental proofs and from the histologic evidence already mentioned, Klose and Vogt drew the conclusion that the spleen is the organ which takes over thymus function when the thymus undergoes physiologic involution.[100] According to their theory the spleen, immediately after birth, is incapable of assuming thymus function, and cannot assume it until a period of preliminary preparation has elapsed, during which its power to functionate grows gradually stronger. If the thymus is removed very early, before there has been time for the preliminary preparation of the spleen to take place, the latter is powerless and the subject dies in a few months; if, on the other hand, the thymus is removed late, after the preliminary preparation of the spleen is complete, the latter organ merely assumes whatever function the thymus may have had and no symptoms develop. If the thymus is removed in the intermediary period—i. e., in the fourth week (from poodles or terriers), although the spleen is not yet fully prepared to take over thymus function, it assumes it perfectly for a time, so that for months the animal remains absolutely normal (the stage of hyperplasia), but, ultimately, on account of its incomplete preliminary preparation during the first four weeks of life, from which it has never succeeded in recovering, it is unable to endure the continued strain thrust on it by the requirements of the growing organism; its functional power begins to fail progressively (the stage of atrophy) and symptoms of thymus insufficiency appear. Finally, its functional power reaches so low a level that the animal sinks into a state of "thymic cachexia" and, having become "idiotic," passes into "thymic coma" and dies a "thymic death." Conversely, Klose and Vogt advanced the theory—as indeed their reasoning compelled them to do, in order to explain why splenectomy in the grown animal is not fatal—that the "retrosternal fat body" takes over the function of the spleen when the latter is removed.

Finally, Klose and Vogt put forward at considerable length[101] a theory of thymus function. According to it in fetal and early postfetal life the thymus is the chief organ of "nuclein synthesis." When

100. Why Klose and Vogt should have arbitrarily selected the spleen for a life and death partnership with the thymus to the exclusion of the pancreas and thyroids is not clear, for their experiments spoke so convincingly for the existence of such a relationship between the thymus and the latter organs.

101. Liesegang was the originator of the theory.

it is removed, phosphoric acid or perhaps nucleic acid is no longer neutralized or combined, and, accumulating, gives rise to a phosphoric acid acidosis. The acidosis is the cause of the alteration in the bones (decalcification) and the edematous condition of the brain, etc. By the aid of this theory and that of a specific reciprocal relationship between thymus and spleen they made to their own minds the apparently divergent effects of thymectomy clear and beautifully correlated into a single whole.

The experiments of Matti appeared in preliminary report[102] in 1911, and in the following year were published in extenso in most complete form.[103] His admirable critical review of the entire subject of thymus physiology and pathology appeared in 1913.[4]

The animals originally used by Matti consisted of eight litters of puppies, containing thirty-six animals, and one brood of four rabbits. The experiments on the brood of rabbits and on one of the litters of puppies (litter No. 8) can quickly be eliminated, for the four rabbits were killed and the six puppies died of pneumonia within a month of the operation. The three thymectomized rabbits showed no abnormalities during life or at necropsy, unless the larger size of the malpighian bodies of the spleen as compared to those of the single control be regarded as such. The dogs in litter No. 8, from which both the spleen and the thymus were removed on the eighteenth day of life, did not die as the result or show any of the train of symptoms so vividly portrayed by Klose and Vogt. Thus, the experimental subjects of Matti, which require careful consideration, become reduced to seven litters of puppies totalling thirty animals, and to twenty-six when we subtract the four puppies (three thymectomized and one control), which were entered in the experiments but died of intercurrent disease before the experiments were completed. Ten of the twenty-six puppies served as control animals, two each for the large litters of from five to seven puppies, and one each for the smaller litters of two.

The age which Matti chose for the removal of thymus in the puppy varied considerably in the different litters. He operated on the dogs of two litters on the eighteenth day of life, but on the dogs of all the other litters when the animals were more than one month old; on the dogs of one litter he did not operate until they were eight weeks old and on those of another litter not until they were twelve weeks old. Thus, all of Matti's thymectomies, except on the

102. Matti, H.: Demonstrationen zur Wirkung experimenteller Ausschaltung der Thymus, Cor.-Bl. f. schweiz. Aerzte **41**:303, 1911.

103. Matti, H.: Untersuchungen über die Wirkung experimenteller Ausschaltung der Thymusdrüse; ein Beitrag zur Physiologie und Pathologie der Thymus, Mitt. a. d. Grenzgeb. d. Med. u. Chir. **24**:665, 1911.

dogs of two litters, were performed at an age when Klose states complete removal of the thymus is "anatomically and surgically impossible," that is, after the fourth week.[104] It is interesting to note, as being at variance with Klose's statements, that of the litters developing a pathologic condition after thymectomy, the litter operated on when the dogs were five weeks old was the first affected, the litter operated on when the dogs were ten weeks old (No. 2) was next affected and the litter operated on the earliest, on the eighteenth day (No. 7) was affected last of all, not developing any symptoms until a "latent period" almost twice as long as the "latent periods" in the case of the other two litters had passed.

Matti observed his dogs for varying periods after thymectomy. Those dogs which developed pathologic changes he killed, in most instances, when the changes were at their height, and those dogs which remained essentially normal were killed when it became clear that nothing further was to be expected from them. Some few dogs he considers that he killed in the early stages of their "thymoprivic" illness. The minimal and maximal observation periods after operation were two and ten months, respectively.

Matti fed his animals at first on milk, later on a mixed diet "appropriate to the age." Exactly how the dogs were kept is not clear. Apparently they were confined in a cellar, but some of them at times were allowed to exercise out of doors. There is every reason to suppose that Matti's animals were well observed.

It is not necessary to describe the operation which Matti employed, for it was the Basch operation with the modification that the thymus was dissected out of the thorax from below upward. Matti did not use any method for the maintenance of artificial respiration.

As already indicated, Matti's experiments fall into two groups, in one of which thymectomy was followed by well-marked changes (experiments on litters Nos. 1, 4 and 7) and in the other by no changes, or by slight changes (experiments on litters Nos. 2, 3, 5 and 6). It must be clearly understood at the outset that the positive results following thymectomy, which Matti reports as the characteristic effects of deprivation of the thymus, were based only on his experiments with litters Nos. 1, 4 and 7 (seven thymectomized and four control animals), and that if he had chosen the other four litters (nine

104. According to the conditions arbitrarily laid down by Klose, Matti's experiments on only two out of eight litters of dogs are valid, because (1) the thymectomies were necessarily incomplete on account of the age of the dogs at the time of operation, (2) the animals were past the period in which thymus has a function of any consequence. Of the two litters thymectomized during Klose's prescribed period, one died before there was time for changes to develop. Therefore, according to Klose, Matti's valid experiments become reduced to those performed on the dogs of one litter (No. 7).

thymectomized and six control animals), as giving the true indications, his conclusions would have been almost directly the opposite of those to which he actually committed himself. The negative or essentially negative results in the majority of his litters and of his animals he explains away for reasons which will be mentioned later. Whether those reasons are adequate or not we shall leave to the reader to determine.

The dogs of litters Nos. 1, 4 and 7 recovered quickly from the operation, and except for an initial setback in growth (Matti attributes this to the immediate effect of deprivation of thymus function rather than to the operation) showed nothing abnormal for the first four weeks (in the case of litter No. 7 not for the first two and one half months) to distinguish them from the control animals. After this latent period had elapsed, however, a definite symptom complex began to manifest itself. The thymectomized animals became less active, showed signs of being more easily fatigued and responded more sluggishly than the control animals. Before long it became apparent that the forelegs of the thymectomized dogs were shorter than those of the control dogs; the lower legs appeared to be particularly short and thick, bowed outward, and the lower radial epiphyses protruded inward. As the bowing increased, the knee joints became displaced further outward and it appeared as if the humeri were rotated inward.

At the same time as the deformities just described were developing in the forelegs changes were making their appearance in the hindlegs. The latter showed signs of weakness and were held at the tibio-femoral and hip joints partially flexed, as in crouching, both when the animal stood and when he walked. The ankle joints bent inward so that the legs touched; the tibio-femoral joints, on the other hand, bent outward. The femur appeared to be rotated outward. As these changes in the legs developed, retardation in growth became increasingly evident; the operated animals were shorter and appeared more stocky. Their hind quarters, however, showed signs of atrophy. In testing the long bones of the legs of the thymectomized dogs at this time, it was found that their rigidity had become greatly reduced (the bones were "federn elastisch").

At this time also muscular weakness made its appearance in the forelegs and in the hindlegs it reached such a degree that the animal supported the weight of the body with difficulty. The movements became trembling. The weakness and bowing of the forelegs before long became so great that the chest sagged down between them to such an extent as to lower the forehead to the level of the knee joints. The forefeet were held in valgus position. The flexion of the joints of the hindlegs correspondingly increased. When the animal stood, all four feet, from ankle joint to toe, were in contact with the ground.

In some of the animals a rachitic rosary could be detected. Growth of the skull was retarded obviously. The weights of the thymectomized animals, which for a time after the initial loss had paralleled the weights of the control animals, began to increase less rapidly. Finally, a point was reached at which the thymectomized dogs ceased to gain altogether or began progressively to lose weight. The muscular weakness at this period became so extreme that some of the dogs could rise from the ground only with help and could not stand at all. The hair became dry and coarse. Decubitus ulcers developed. Natural death would soon have intervened. Matti, however, sacrificed all of his animals reduced to this helpless state except one. That animal died.

While the symptoms just described were developing, Matti was unable to detect any signs of diminished intelligence or perversion of appetite such as described by Klose and Vogt.

In the blood of the thymectomized dogs Matti found no changes, except a secondary anemia and a delay in the physiologic decline of the lymphocytes and the increase in the polymorphonuclear leukocytes.

We shall not describe the pathologic condition which Matti found in the bones of those of his animals showing the symptoms just enumerated other than to say that it was rickets or a very closely related condition, exactly as Matti considered it to be. Characteristic disturbances in endochondral ossification, excessive osteoid production, fractures, partial and complete, were all present. Indeed, the disease in some of Matti's dogs, as indicated both by his descriptions and illustrations, seems to have reached a severity rarely seen in infantile rickets and to have equalled in the resulting weakness and distortion of the skeleton the severest forms of osteomalacia.

In addition to the pathologic changes in the skeleton, Matti reported definite alterations in the muscles, suprarenals, thyroids, spleen, pancreas and—in the only dog examined—in the nervous system. In the muscles Matti found in places diminution in size of the muscle fibers and absence of cross striation, increase in both muscle and connective tissue nuclei, increase of fat between muscle fibers, etc. Matti explained these changes as the result, in part, of inactivity of the muscle, in part of deprivation of thymus function. The suprarenals showed hypertrophy of the medulla and in most instances, but not in all, a more marked reaction to the salts of chromium and a grouping of cells similar to that described by Störk and Haberer[105] and interpreted as indicating hyperplasia. The thyroids of the thymectomized group were relatively a little larger than those of the control animals and composed of more densely arranged tissue; the alveolar epithelium was higher and thrown into folds; the colloid was diminished and

105. Störk, O., and Haberer, H.: Beitrag zur Morphologie des Nebennierenmarks, Arch. f. mikr. Anat. **72**:481, 1908.

desquamation of the alveolar cells was frequently encountered. The hypophysis showed a moderate, relative hypertrophy, which was not present when the animal became "cachectic." The spleens of the thymusless animals contained more numerous and larger malpighian bodies than those of the control animals. The pancreas was hypertrophied and the impression was obtained that the islands of Langerhans in the tail were the more numerous in the thymectomized dogs. The brain and cord of the only thymectomized animal examined showed changes similar to those described by Klose and Vogt.

Although the pathologic changes in the different organs just enumerated were regarded by Matti as characteristic developments after thymectomy, yet a perusal of the protocols of his experiments on the positively reacting litters (Nos. 1, 4 and 7) indicates that even among them the lesions were not in all instances so clearcut and distinctive as Matti's general description indicates. For example, the spleens of the two thymectomized animals in litter No. 7 were distinctly aplastic, and in litter No. 4 the spleens of thymectomized and control animals showed no essential differences. The histologic changes in the thyroids of some of the thymectomized animals seem to have been shared, to an extent at least, by the thyroids of some control animals. The differences reported in the pancreatic glands and hypophyses become negligible on analysis. The lesion in the bones appears, however, to have been definite and constant in the animals of all three litters, and is the essential pathologic condition in Matti's experiments.

Although the four control animals in litters Nos. 1, 4 and 7 remained relatively little affected, while their thymectomized mates developed the severe condition described, yet two of them at least showed definite changes in the skeleton of somewhat the same nature as those which appeared in the thymectomized animals. For example, the femurs of the control dogs, Nos. 11 and 12 of litter No. 4, showed "slight irregularities in the process of endochondral ossification," "broadening in places of the cartilaginous zone," "localized areas of osteoid formation in the metaphyses," and some irregularity in the growth of the sprouts of the marrow vessels into the cartilage. The control dog in litter No. 7, which survived to the period when the thymectomized animals developed changes, showed "slight bending of the distal radial epiphyses."

Of the four thymectomized litters which failed to develop the remarkable symptoms and lesions just recorded, Matti states that one litter (No. 3) showed definite signs of reacting positively, one litter (No. 2) showed suspicious signs, one litter (No. 5) showed signs apparently positive—if one experimental animal and one control animal were excluded—and one litter (No. 6) showed no signs of positive reaction whatsoever. But the protocols of the experiments on these

four litters do not substantiate even these moderate claims. The thymectomized animals in them showed no retardation in growth as measured in terms of weight. Though some of them were lighter others were heavier than their control dogs.[106] None of them showed a single abnormality of the skeleton. It is a striking and important fact that not one of the thymectomized animals in these four litters showed pathologic changes equal to those of the two control dogs of the positively reacting litter No. 4. Indeed, the only differences recorded seem to have been in the thyroids and suprarenals and may be summarized by the statements that the thyroid of the thymusless animals seemed to show a greater tendency toward the hyperplastic type, and that in like manner the width of the medulla of the suprarenal appeared to be greater among them.

The reasons which Matti advances in justification of his selection of the results of his experiments on litters Nos. 1, 4 and 7 as being the true or typical results of thymectomy, and of his exclusion of the results of the experiments on the other four litters as not being the true or typical results, are remarkable and important; we quote them in their entirety.

"For the elucidation of the negative experimental results following thymectomy and of the greater effects of thymectomy in younger animals the following conditions, in our judgment, present themselves for consideration. According to Groschuff, there is present in many of the mammalia, including the dog, a thymus rest derived from the fourth pharyngeal pouch, the presence of which can be discovered only by complete serial section of the neck. It is reasonable, therefore, to connect experiments having a negative outcome with the existence of the functionally active rest from the fourth pouch. The fact that the entire litter and never, in our experiments at least, single members of it behaved in a refractory manner to excision of the thymus can be interpreted certainly as speaking for this view. Further, it must be remembered that there are organs closely related to the thymus in their

106. At the end of Matti's experiments on these four litters the control in experiment No. 2 was 2.3 kg. lighter than the thymectomized animal; in experiment No. 3 the control was 1.5 kg. heavier; in experiment No. 5 one of the two control animals (No. 17) dying of distemper, weighed 450 gm. less than his thymectomized mate (No. 14), which was killed in a healthy condition. (Three weeks previously when both animals were in normal health the control [No. 14] weighed 500 gm. more than the thymectomized animal.) The other control in this litter (No. 16) was approximately 2 kg. heavier than either of her two mates; in experiment No. 6 one of the two control animals (No. 23) was the lightest of all six animals in the litter, and the other control (No. 22) was 3.5 kg. heavier than one thymectomized animal (No. 21), exactly equal in weight to another (No. 18), lighter by 2 kg. than another, and lighter by 4 kg. than the remaining thymectomized dog. The heaviest animal in this litter, operated dog No. 20, weighed 20 kg., whereas the lightest animal, control dog No. 23, weighed only 12.3 kg.

activity, particularly when considered from the standpoint of the symptoms caused by their removal . . . and that between the thymus and these related organs exactly as between other organs of internal secretion correlative relationships exist, and it is possible that these related organs can, when the need arises, vicariously functionate for the thymus. . . . In any event, in these ways it becomes clear why deprivation of thymus function does not, in each experiment, cause symptoms of thymus insufficiency and why these latter do not always manifest the same degree of severity. That variations within physiologic limits here exist is a priori altogether likely. . . . The consideration of the conditions which have been set forth offers, in our judgment, a satisfactory and complete explanation for the negative or weakly positive experiments in extirpation of the thymus." [107]

Matti concluded from his experiments that the thymus was an organ of internal secretion, essential to life, and that its removal produced profound alterations in bodily growth, in the formation of bone through some interference in the intermediary metabolism, in the muscles and caused the changes in the suprarenals, thyroid and other organs which have been recorded. He considered that the function of the thymus was bound up with those of other endocrine glands, particularly with the thyroid, and the medullary portion of the suprarenal. If any organ of internal secretion could be spoken of primarily as an organ of growth (Wachstumsorgan), it was the thymus.

The arguments which Matti uses for eliminating the results of his "negative" or "weakly positive" experiments, as not representing the

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107. Für die Erklärung der negativen Versuchsergebnisse und für die grössere Wirksamkeit der Thymektomie bei jüngeren Tieren kommen unseres Erachtens folgende Umstände in Betracht: Nach Groschuff kommt bei vielen Säugern, auch beim Hund, ein Thymusmetamer IV vor, über dessen Vorkommen wir uns nur an lückenlosen Halsserien orientieren können; es liegt nun nahé, negativ ausgefallene Versuche mit der Existenz funktionstüchtiger Thymusmetamere IV in Verbindung zu bringen. Dass, weningstens in unserem Material, gegebenen Falles stets der ganze Wurf, und niemals nur einzelne Glieder desselben sich gegen die Thymusexzision refraktär verhalten, könnte wohl für diese Auffassung sprechen. Ferner ist zu bedenken, dass es Organe gibt, welche in ihrer Wirkung, entsprechend auch hinsichtlich ihres Ausfalls, mit der Thymus nahe verwandt sind, wie noch näher zu besprechen sein wird, dass zwischen der Thymus und diesen verwandten Organen sowie anderen Drüsen mit innerer Sekretion offenbar korrelative Beziehungen existieren, und dass diese Verhältnisse vikariierendes Eintreten verwandter Organe für die Thymus möglich erscheinen lassen. . . . Jedenfalls wird aber auf diese Weise erklärlich, warum der Ausfall der Thymusfunktion nicht in jedem Experiment Ausfallsymptome und auch nicht immer gleich starke Ausfallsymptome nach sich ziehen muss. Dass hier physiologische Schwankungen vorkommen, ist a priori recht wahrscheinlich. . . . Die Berücksichtigung der dargelegten Verhältnisse bietet unseres Erachtens eine genügende und befriedigende Erklärung für die negativ oder nur schwach positiv ausgefallenen Versuche experimenteller Thymus-ausschaltung[169] (p. 803).

true effects of deprivation of thymus function, are of great importance, not only in their bearing on the conclusions to be drawn from his own work, but in their general applications. If the reasoning involved in them is valid, they deprive of all value all negative results following removal of any suspected organ of internal secretion and, indeed, make devoid of significance all negative experiments in the field of endocrinology. Stated briefly, with the implications involved in them, they are as follows: In those dogs, partially or completely refractory to thymectomy, either there is still present a rest of thymus tissue, hidden from the naked eye, so minute as to be capable of demonstration only by means of a faultless series of serial sections of the entire neck, yet so endowed with function as to be able to perform alone what was formerly accomplished by the entire gland, or else there exist in the other organs of internal secretion peculiar properties, entirely lacking in animals reacting positively to thymectomy. enabling them, on demand, instantly to replace the thymus with such perfection that the sudden destruction of that gland, though "essential to life," creates scarcely a stir on the surface of the stream of normal development.

Though we cannot deny in our present state of ignorance that other organs of internal secretion may possess the power to assume the function of the thymus when the latter is destroyed, we are not free to assume that possibility to be an established fact and proceed to use it as a means of disposing of experimental results which we do not like. Indeed, before we could assume it, proofs as definite as those demanded for the existence of thymus function itself, would be required. Not only are such experimental proofs at present lacking, but whatever evidence thymus extirpation affords points away from that supposition. For if certain endocrine glands in animals refractory to thymectomy do actually exercise increased function as soon as the thymus is removed, they should show morphologic evidence of it; i. e., they should be hypertrophied or hyperplastic or show more of the finer cellular changes indicative of increased functional activity. But it has been the almost universal experience of those who have studied the effects of thymus removal that animals, remaining normal after thymectomy so far as gross changes are concerned, show little or no alterations in the endocrine glands, whereas those animals showing the most marked alterations in growth and in the skeleton are also the ones that exhibit the most marked pathologic conditions of the organs of internal secretion. Matti's own experiments and those of Klose are splendid illustrations in point.

Matti's alternative hypothesis cannot be tolerated. Because the experiment does not turn out as he thinks it should, he supposes that a thymus rest is present and discards the result. He holds the negative results of other investigators to be invalid because they are unable to give absolute proof that no thymus rests existed in their thymectomized

animals, but he feels free to cast aside at will his own negative experiments on the ground of imaginary thymus rests when he has endeavored by all the means at his disposal to demonstrate their existence and failed to do so. The reason which he gives for thinking that a concealed thymus rest must have been present in his refractory animals, because whole litters failed to react, rather than single animals, is, in reality, no reason at all. Perhaps the pathologic condition which he encountered was due to a disease, for example, rickets, wholly unrelated to destruction of thymus and certain litters remained exempt because of familial insusceptibilty, etc. On this particular point, however, Matti is "hoisted by his own petard."

"On account of the well known intense regenerative capacity of thymus rests which are left behind at operation, to which Basch has already called attention, incomplete extirpations of the thymus can be blamed for the negative outcome of certain experiments" . . .[108]

"The demand for complete extirpation of the thymus is grounded on the well known intense regenerative capacity of the thymus, first recognized by Friedleben."[109]

If, then, the thymus rest left at operation possesses this "well-known intense regenerative capacity," why was Matti unable to find it or any trace of it in the form of a thymus fat body (Fettkörper) in any one of his nine refractory animals? "The wide exposure and the complete view which the method of operation chosen by us ensured permitted a complete removal of the thymus. Furthermore, we made certain at the necropsy of complete removal of the organ, and as a final measure, by microscopic examination of the fat which was present. *A true thymus fat body was never present.*"[110]

It stands to reason that if no thymus rest can be found in a thymectomized animal, then it is necessary to consider the thymectomy complete and the experiment valid, and to abide by the results obtained and to continue so to do until the rest is found, though it require a "faultless series of serial sections of the entire neck" to accomplish this end. We do not think that Matti can possibly shake himself free from the shackles of his negative experiments.

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108. "Bei der bekannten intensiven Regenerationsfähigkeit zurückgelassener Thymusreste, auf die schon Basch aufmerksam machte, könnten auch unvollständige Exstirpationen am negativen Ausfall gewisser Experimente schuld sein"[103] (p. 804).

109. "Die Forderung totaler Thymusausschaltung wird begründet durch die bekannte, schon durch Friedleben nachgewiesene intensive Regenerationsfähigkeit der Thymus"[4] (p. 60).

110. . . . "Der breite Zugang und die vollständige Uebersicht, welche die von uns gewählte Methode gewährte, erlaubten stets eine vollständige Entfernung der Thymus, und zudem überzeugten wir uns bei der Autopsie stets von der vollständigen Entfernung des Organs, eventuell auch durch mikroskopische Untersuchung vorhandenen Fettgewebes; ein eigentlicher 'Fettkörper' war nie vorhanden"[103] (p. 804).

The conclusions which Matti drew from his positive experiments might have been modified, in our opinion, in view of his negative experiments, in some such way as follows: Though in some animals the thymus gland appears to be essential to life, in others, of the same age, species and breed, it appears to be absolutely unessential; although in some removal of the thymus is followed by the most profound disturbances in growth and the development in the bone of a pathologic condition identical with rickets, it is followed by no such disturbance in growth and no alterations in the skeleton in others; though removal of the thymus in some animals is followed by alterations in certain of the organs of internal secretion, notably the thyroid and suprarenals, in others, indistinguishable from them, it is followed by no changes, or by changes comparatively slight.

Although we have disagreed with Matti in regard to the interpretation of his negative experiments and, therefore, of his work, nevertheless, we wish to express admiration for its painstaking and excellent character. Whether Matti's conclusions are correct or not, no finer attempt has been made to solve the problem of thymus function.

As the result of the reexamination of his material in the light of Matti's findings, Klose came to the conclusion that the lesion in the bones of his thymectomized animals was rickets, and, indeed, when once its true nature was revealed, it appeared to him so typical and well marked as to cause him to state that removal of the thymus was the only method by which a rickets, almost identical with human rickets, could be produced in animals ordinarily not subject to the disease, provided the diet was normal. The responsibility for the former diagnosis of osteoporosis he shifted onto Recklinghausen, and cited Aschoff in support of the new view.

It is interesting to note that the osteoid borders of the trabeculae formerly described as "diminished" became broad in the revised report, and that the cysts[111] "found everywhere," "many larger than hazelnuts," and which were said to have been the cause of the spontaneously occurring fractures, are not even mentioned in the new description and are not shown in the new illustrations, either the radiographs or plates, although they were exhibited in the old by both drawings and roentgen ray.

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111. ". . . the spontaneous fractures are found, in each instance, at the site of the largest cysts (Fig. 29, a, b). Many are larger than hazel nuts. They are irregular; the small ones, which are visible with the naked eye, have a more nearly round shape; the large ones are elongated and often irregular. When longitudinal sections of the bones are examined with a dissecting microscope, small, scarcely visible cysts can be seen, which are distributed everywhere, but are especially numerous in the metaphysis. An occasional cyst may be found in the epiphysis"[82] (p. 68).

We shall not state Klose's revised version of his findings in the bones of his animals; in substance, his new description is identical with the description given by Matti of the lesions in his animals.

Although Klose had stated definitely in his previous papers that there were no histologic changes in the thyroids of his thymectomized dogs, yet, after Matti reported definite changes in the thyroid in his experiments, Klose made the discovery that in reality the same changes had been present in his animals also. Indeed, the reader is made to infer that, once they were noticed, they became exceedingly well defined and were present only in the thymectomized animals, never in the control animals. They consisted of diminished colloid, cubical or cylindrical epithelium, infoldings, desquamation and enlargement of the follicles.[112]

So, too, after Matti had described hypertrophy of the medulla of the suprarenals and accompanying histologic changes, Klose made the discovery that the same condition existed "without exception" in the suprarenals in his thymectomized animals.

Klose also brought to light changes in the muscles similar to those described by Matti.

Whether or not Klose was influenced by the criticisms of Matti and others of his theory of a specific functional interrelationship between thymus and spleen, he withdrew it entirely in his 1914 article,[92] stating that the experiments of Flesch had "taken the bottom out of it." He also modified some of his more important former statements in regard to the symptoms which his dogs had shown after thymectomy. For example, he states in the 1914 article that not all but "many" of the thymectomized dogs showed an altered state of intelligence, which not always but "occasionally" could be spoken of as idiocy, and that the terminal state of "thymic coma" occurred not invariably but "often." In this article he also remarks that the operation must be done on "young" animals, dismissing the subject of age limitation for successful experiment without alluding to his former remarkable statements. But in spite of Matti's sharp criticisms Klose clings to the theory that the thymus is the organ of "nuclein synthesis" and on the analogy of a gelatin experiment in vitro continues to attempt to explain the changes in the bones, edema of the nervous system, etc., by means of it.

Klose's 1914 publication[92] is not, however, entirely a reconstruction of his former findings, for it contains some minor results of thymectomy not previously reported, namely, that loss of thymus causes retardation in the time of eruption and an underdevelopment of the permanent teeth, and that in three thymectomized dogs there was an

112. Matti described diminution in size of the follicles and Klose's illustrative plates would indicate diminution in size.

absence of the hydrochloric acid cells of the stomach and that extensive examinations of the blood showed a slow and progressive decline in the number of lymphocytes.

The results which Klose reported in this same year (1914) from thymus extirpation in the pig, goat and rat[113] are as follows:

The pig must be operated on in the fourth or fifth week of life(!). The rachitic changes appeared on the average within from two to three months and consisted of rachitic rosary, bending of the extremities, spontaneous fractures and loss of power to walk.[114] The gland cannot be extirpated completely from the pig and when the small rest left "completely regenerated" to the size of the original gland, the signs of rachitis disappeared. The results following thymectomy in the goat were "analogous" to those in the pig. In the goat the gland must always be taken out in the fourth week (!).

It is difficult to know what valuation to put on the work of Klose, one reason being that that investigator has omitted from the reports of his experiments almost all detailed information concerning them. The reports of his experiments are composed almost entirely of generalizations or, better, stock descriptions of the results obtained. Remarkable as it may appear, in not one of Klose's elaborate publications is there a description, however brief, of a single thymectomy experiment. Since then, Klose does not permit his readers to gain insight into the character of his work, the acceptance of it becomes a test of faith in Klose as an investigator.

In the first place, Klose's alteration of some of his previously published experimental results under the stimulus of Matti is not creditable to him as an observer and impairs our confidence in the value of an experiment done by him. Experimental results capable of being revised once can perhaps be revised again. In the second place, it is difficult not to think that Klose has failed to make a complete and frank statement of all the results of his experiments. The picture which he paints of the effects of thymectomy is altogether too perfect to have represented all of his actual experiences.[115]

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113. Klose makes no mention of the results of his thymectomy experiments in the fowl, which he states were conducted at the same time as those on the pig, rat and goat. We have no information about Klose's experiments on the pig and goat except his general statement of the results and almost none about his experiments on the rat.

114. Aschoff states that Klose's thymectomized pigs showed no changes except in some instances slight retardation in growth[8] (p. 131).

115. Our suspicions appear to have some actual foundation. In the reports of his experiments published prior to 1914, Klose states that he operated on fifty-four dogs in succession without a fatality and gives his readers clearly to understand that in that series of fifty-four experiments there was not a single failure and not a single exception in any essential particular in the results obtained. In his 1914 publication on the effects of thymectomy in the rat, goat and swine,

Lastly Klose exhibits such predilection for the bizarre and extravagant, for exaggerated statement and categoric affirmation and denial on wholly insufficient grounds, for the use of hypothesis as if it were established fact; in brief, he shows such general disregard for the logical and a lack of sense of proportion, that it is difficult to imagine that his observations were purely objective and his judgment impartial and conservative in the field of experiment.

We shall limit ourselves to a few comments on some of the more important of Klose's experimental results. Comparing the descriptions and drawings of the lesions in the bones which appear in the Klose-Vogt publication of 1910 with those in the 1914 publication, it becomes certain that the pathologic condition in the bones of Klose's dogs could not possibly have been a uniform one.[117] If it was rachitic in some experiments (1914), it was no more than osteoporosis in others (1910-1912). It seems fair to assume from Klose's description and illustrations that, if rachitis was present in the Klose-Vogt series of fifty-four dogs, it was much the exception.

The degenerative changes in the nervous system of the thymectomized animals described by Klose do not appear to have been greater than those found not infrequently in dogs dying after illnesses from a variety of causes. Because they were present in the thymectomized animals, which had passed through "stages of cachexia" and "coma," it in nowise follows that they were caused by deprivation of thymus.

The atrophic condition of the spleen found regularly in the "stage of cachexia" has no significance whatsoever in so far as its bearing on

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however, there occurs this sentence: "At first the investigations were productive of a negative result; the rachitic manifestations were only transitory. The reason that many litters behave in a refractory manner lies in the fact that, *just as in many breeds of dogs,* well developed cervical portions of the thymus are present in the thyroid gland . . . which gradually undergo functional atrophy, as I have been able to recognize in thymectomized animals."

"Zunächst ergaben die Untersuchungen ein negatives Resultat, die rachitischen Erscheinungen waren nur vorübergehend. Der Grund dafür, dass sich manche Würfe refraktär verhalten, liegt ebenso wie bei manchen Hunderassen darin, dass neben dem vorzugsweise thorakalen Hauptthymus noch stark ausgebildete zervikale, in der Schilddrüse gelegene Thymusteile entwickelt sind, die, wie ich an den operierten Tieren nachweisen konnte, allmählich funktionell hypertrophieren[116] (p. 4).

116. Klose, H.: Thymusdrüse und Rachitis, Centralbl. f. allg. Path. u. path. Anat. **25**:1, 1914.

117. Compare the microscopic preparation of the epiphyseodiaphyseal junction of the femur shown in Fig. 7, Table 4 (1910), with the microscopic preparations of ribs and femur in Figs. 18, 20 and 21 (1914). The former (Fig. 7) certainly may be considered the most typical and representative example of the lesion which Klose's material in 1910 showed, for it is the only preparation exhibited. It is as clearly not rachitis as the preparations of 1914 are rachitis. It may be assumed that such distinguished authorities on bone pathology as Recklinghausen and Aschoff did not express different opinions without reason.

Fig. 7.—The kennels and inclosures in which the greater number of the animals of the experiments were kept.

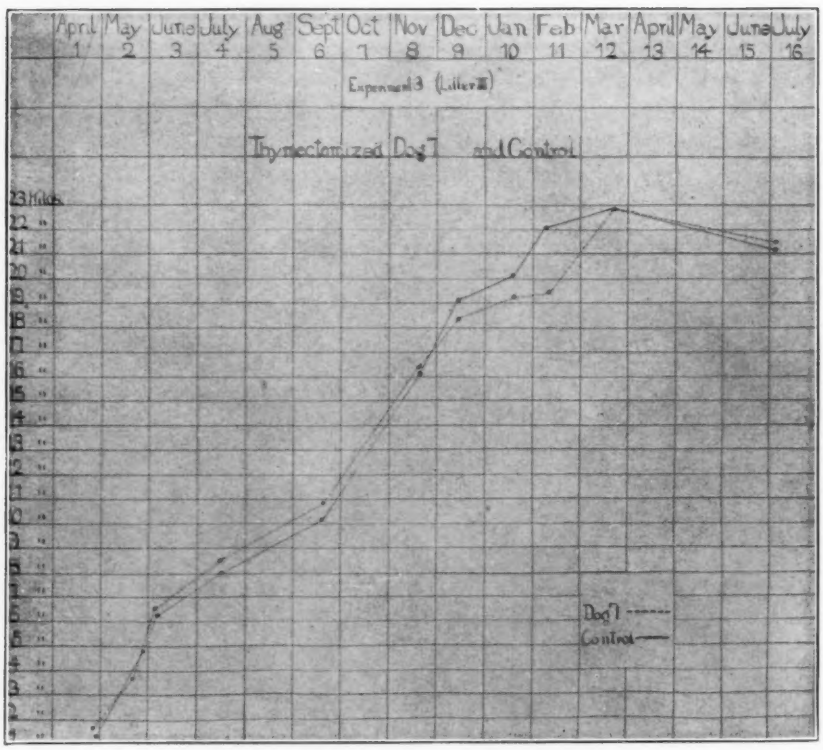


Fig. 8.—Weight curves of dogs Nos. 7 and 7, control, Exp. 3.

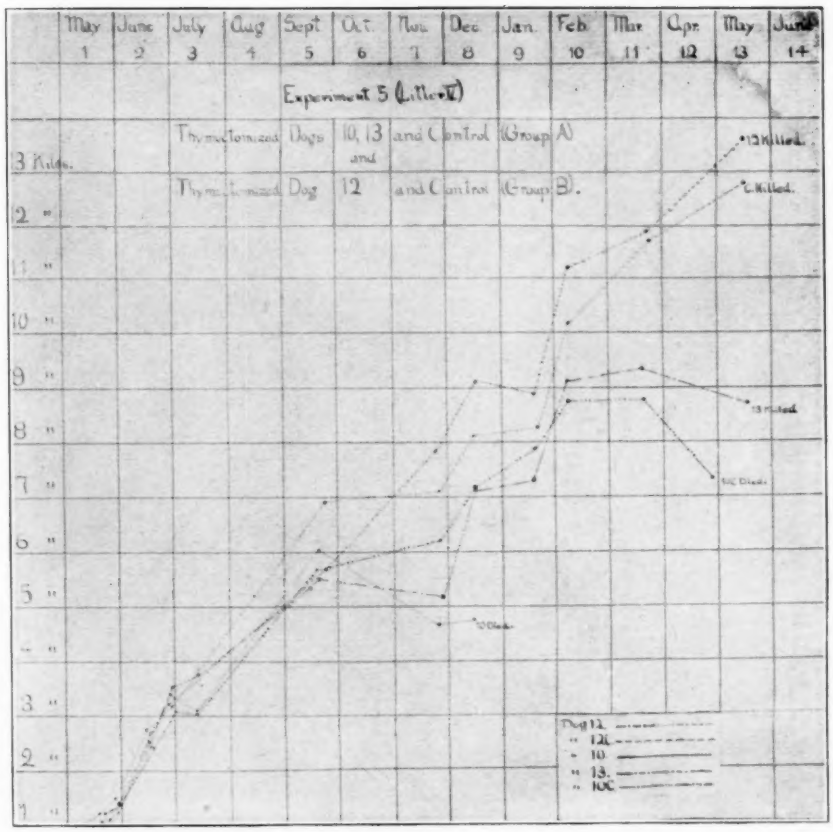


Fig. 9.—Weight curves of dogs Nos. 10, 13, 12, 10, control, and 12, control, Exp. 5.

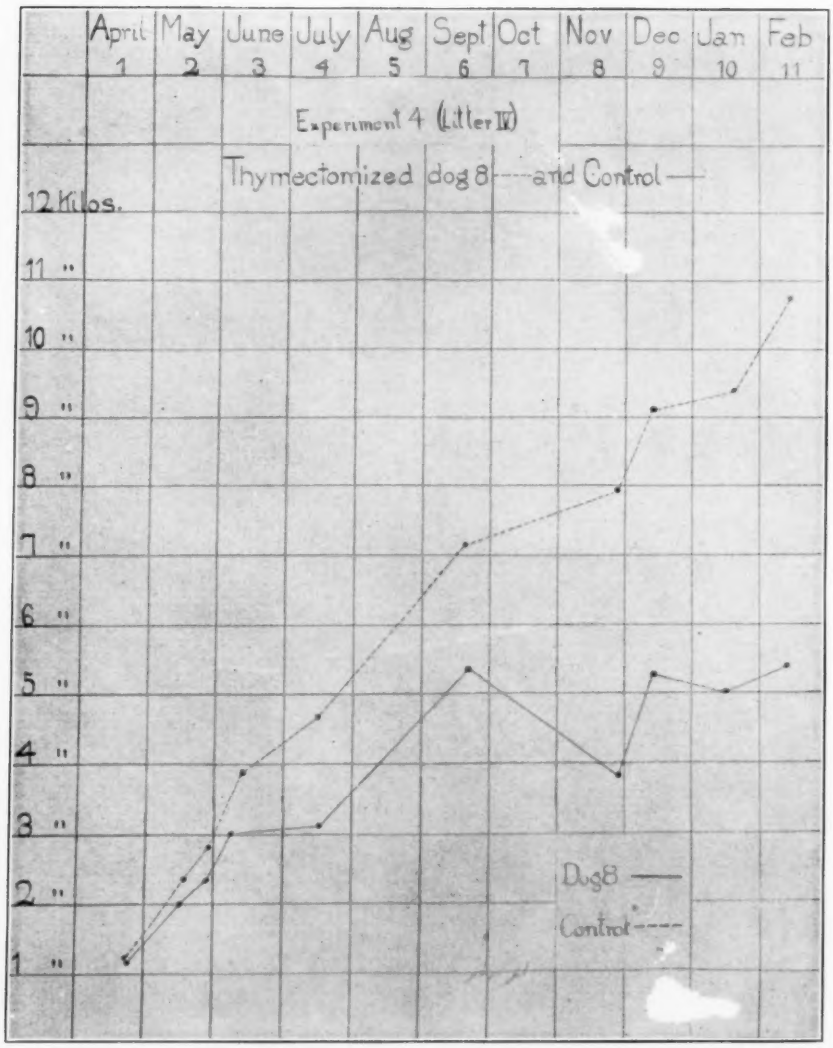


Fig. 10.—Weight curves of dogs Nos. 8 and 8, control, Exp. 4.

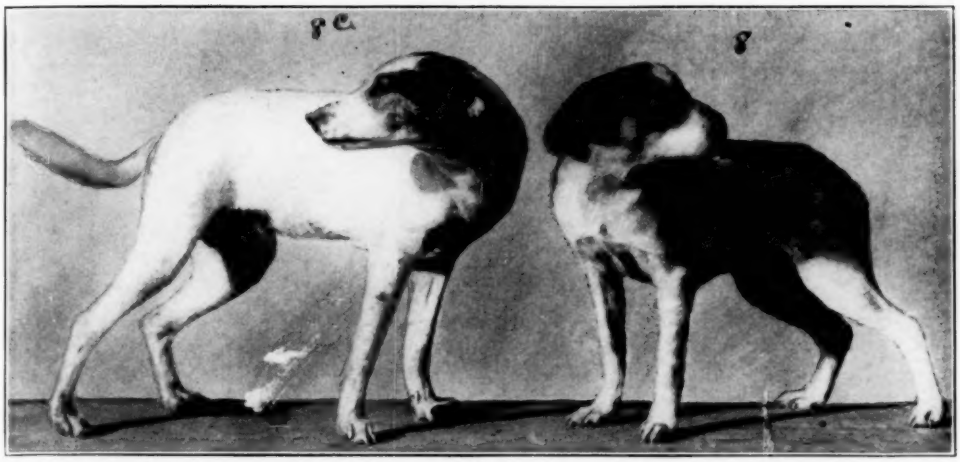


Fig. 11.—Thymectomized dog No. 8, and control dog No. 8, Exp. 4, photographed when 8 months and 4 days old. Period of observation, seven months and fourteen days (background removed). Note that dog No. 8 is more slender and smaller than his control mate but is normally formed.

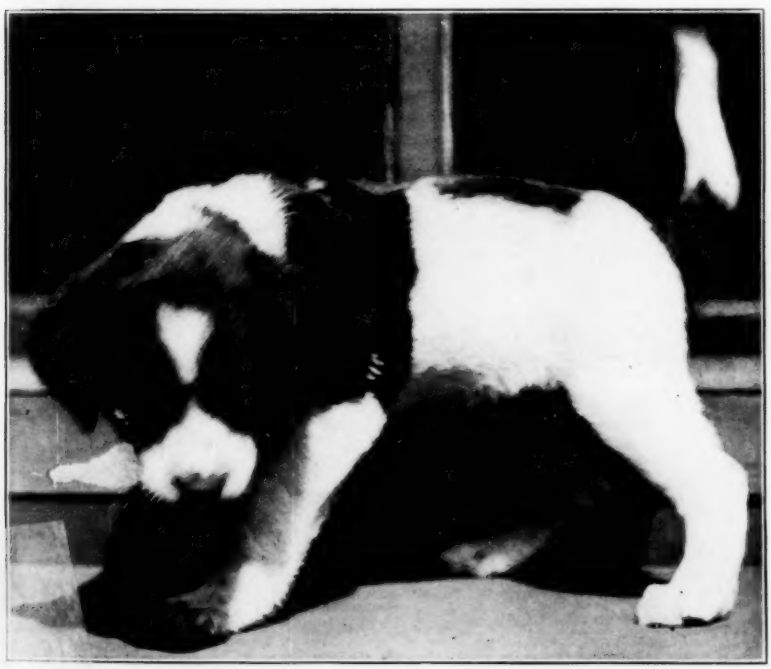


Fig. 12.—Thymectomized dog No. 8, Exp. 4, photographed when 2 months and 4 days old; one month and fifteen days after operation.

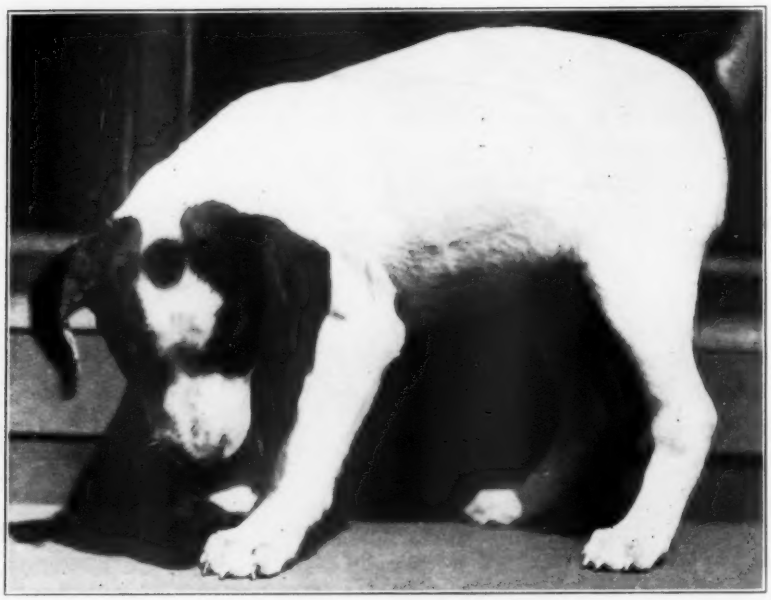
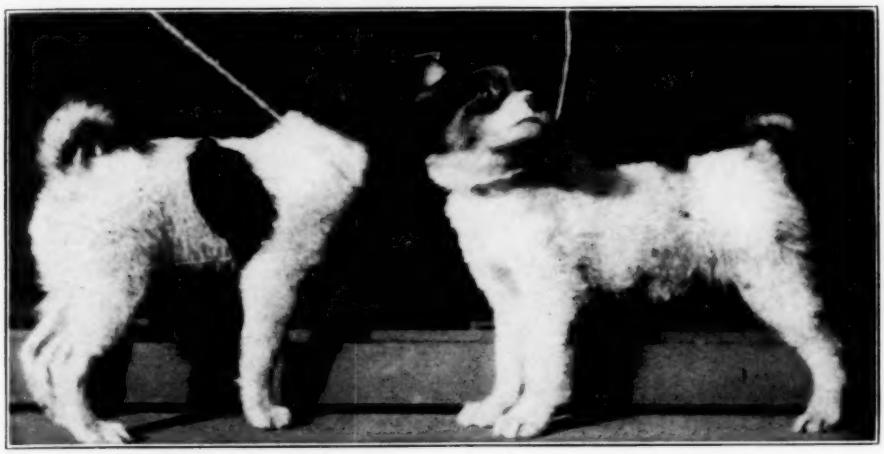


Fig. 13.—Control dog No. 8, Exp. 4, photographed when 2 months and 4 days old. Period of observatoin one month and fifteen days.



39 39C

40C 40

Fig. 14.—Thymectomized dog No. 39 and control dog No. 39, thymectomized dog No. 40 and control dog No. 40, Exp. Photographed when 3 months and 27 days old. Period of observation, three months and eight days. Note that thymectomized dog No. 40 is considerably larger than No. 40, control. Dogs No. 39 and No. 39, control, are about equal in size. Neither thymectomized animal shows any abnormality.

39C

39

40

Fig. 15.—Thymectomized dog No. 40 (No. 40 C died from distemper), No. 39 and No. 39, control; Exp. 8. Photographed when 14 months and 8 days old. Period after operation, thirteen months and nineteen days. Thymectomized dogs Nos. 39 and 40 show no abnormalities.

loss of thymus function is concerned. Atrophy of the spleen is the regular accompaniment of "cachectic" states in dogs.[118] The hyperplasia of the spleen in the "stage of adiposity" might have had significance if of definite and invariable occurrence. But the spleen of the dog like the lymph nodes is subject to the most marked variation and the greatest care should be exercised before concluding that a pathologic condition in it is to be attributed to loss of thymus function. The importance of the changes in the suprarenals overlooked by Klose until 1914, and those in the thyroids looked for but not seen, we shall discuss in connection with our own work.

The time limitations arbitrarily set by Klose for complete thymectomy and for the production of the characteristic clinical and pathologic manifestations appear on the face of them to be improbable; for example, that the thymus can be removed completely from the dog in the fourth week, but not by any possibility in the fifth week, and that removal in the third week inevitably causes death but in the fifth week never more than transitory symptoms. We should like to know on what experimental evidence these sweeping statements of Klose's rest, and what foundation he has for such assertions as that poodles and terriers are far more susceptible than other dogs to loss of thymus, for we should suppose a much larger series of experiments would be required in proof of them than the series used by Klose to determine the gross effects of extirpation of the thymus gland. We wish to call attention to the fact that by means of his arbitrary age limitations for successful experiment Klose excludes by far the greater part of the experimental results which are not in accord with his own. We cannot find that he has failed in a single instance, however, to accept without question *positive* results in any way resembling his own, irrespective of the age of the dog at the time of thymectomy or, we might add, the mode of operation or conduct of the experiment.

The disturbed state of intelligence described by Klose in his thymectomized dogs does not seem to have exceeded or to have differed from that seen not infrequently in extremely sick animals. We doubt whether Klose had the slightest justification for regarding it as "idiocy" or as a specific condition dependent on loss of thymus function.

Klose's hypothesis of interrelationship between thymic and splenic functions we need not discuss, since he has withdrawn it. We might remark, however, that a better example of a *reductio ad absurdum* could scarcely be found than Klose's exposition of his own theory, leading to the inevitable conclusion that the "retrosternal fat body" possesses the power to take over the function of the spleen when the

118. Compare authors' experiments.

latter is removed. Though he has withdrawn his theory, the experiments on which it was based still stand pointing to its truth with the same force as formerly.

In regard to Klose's theory of explanation of thymus function based on the supposition that the thymus is the organ of "nuclein synthesis" we point out merely that it rests on speculation alone and not on one single valid argument. It is surprising that from 1910 to 1914, only theoretical support was brought forward in its favor, when it was possible at any time to determine its truth by chemical examination of the blood of the thymectomized animals.

Gebele,[119] made complete extirpations of the thymus in nine dogs and a partial extirpation (one half of the gland) in one dog. Of his completely thymectomized dogs one died from the anesthetic, three from hemorrhage, one survived only three days (infected wound) and one lived but fifteen days (infected wound). Of the remaining three animals, one died at the end of twenty-two days (severely infected with fleas) but was not necropsied so that no conclusion in regard to the effects of thymectomy was possible in that animal; another, weighing 1.5 kg. at the time of operation, survived for one and one half months, growing faster than the control animal; the last dog, in which thyroids and parathyroids as well as thymus were said to have been removed, lived two months and eleven days.

In view of the character of Gebele's experiments here indicated, the description which he gives of the results becomes truly remarkable.

"The dogs remain alive after total thymus extirpation. Yet, they developed changes, walking with a straddling gait and flat footed; they grew tall and looked long legged. The nutrition of the thymectomized animals was equal to or, in some instances, better than that of the control dogs. But at the end of about three months they became fat and pudgy (schwammig) and began to emaciate progressively. At the same time they became sluggish and the bones became flexible." [120]

Hart and Nordmann[121] removed the thymus gland from a series of puppies (they do not state the number) between the second and the fifth weeks of life, employing an operative method which differed from that of all other investigators. They entered the thorax by cutting the

119. Gebele: Ueber die Thymus persistens beim Morbus Basedowii; klinisch-experimentelle Studie, Beitr. z. klin. Chir. **70**:22, 1910.

120. "Bei der totalen Thymusexstirpation bleiben die Tiere am Leben. Doch veränderten sich die Hunde, sie wurden im Gang breitspurig, traten plat auf, schossen im Ganzen in die Höhe und sahen hochbeinig aus. Der Ernährungszustand der Tiere war dem der Kontrolltiere gleich, zum Teil über. Die Tiere wurden fett und schwammig, um nach Ablauf von ca. 3 Monaten mehr und mehr abzumagern. Dabei stellte sich Apathie ein, die Knochen wurden biegsam" (p. 60).

121. Hart, C., and Nordmann, O.: Experimentelle Studien über die Bedeutung der Thymus für den tierischen Organismus, Berl. klin. Wchnschr. **47**: 814, 1910.

bony ribs on the left side from the first to the fifth, presumably at points near the costochondral junction and, lifting the sternum with its attached cartilages and rib ends upward and to the right, obtained an exposure of the thymus which enabled them to dissect it out of the thorax under direct vision, but at the cost of a left sided pneumothorax. In those animals which died shortly after the operation they were unable to find a trace of thymus. They fed their dogs a mixed diet and kept them in a large garden plot where they could run about. They controlled from the same litters. They observed that the operation affected some of the puppies so severely that they did not entirely recover for a number of weeks. Nevertheless, they thought that, quite apart from the effects of the operation, the growth of the thymusless animals was retarded, for the latter never caught up with their control mates in spite of enormous appetites. They noticed also that the thymectomized dogs, some weeks after the operation, developed coarseness of the hair and pyodermia, which latter could be cured but tended constantly to recur, and that the thymectomized dogs were less vigorous than the control animals, becoming easily fatigued, and had less firm muscles. All of the thymectomized animals died before the end of a year and at necropsy all showed enormous dilatation of the heart with pallor of the heart muscle. In the testes of the thymectomized animals Hart and Nordmann found an increase in the numbers of interstitial cells and an atrophy of the sperm cells and absence of signs of spermatogenesis and somewhat analagous, but less definite, changes in the ovaries. They could not find changes in the thyroids, adenoid tissue, nervous system or bones. There was not the slightest trace of rachitis and the length of the bones of the thymectomized animals was not less than of the control animals.

Hart and Nordmann also made some thymus transplantation experiments into animals with intact thymus glands and observed diminution of appetite, emaciation, etc., which were transitory in character.

They concluded that the thymus is an important and perhaps essential organ during the period of growth, having a close connection with metabolic processes of the body and the regulating mechanism of cardiac activity and probably having something to do with the resisting powers of the body against bacterial infections. Their somewhat extended theoretic explanation of the occurrence of the dilatation of the heart, based on the interruption of the normal interaction between thymus and suprarenal, we shall not discuss.

Nordmann[122] later reported further experiments in thymus extirpation, performed by Hart and himself, this time on dogs from 3 to 6 weeks old and in some younger animals (again the authors do not state

122. Nordmann, O.: Experimentelle Studien über die Thymusdrüse, nebst Bemerkungen zu der Meltzerschen intratrachealen Insufflation, Arch. f. klin. Chir. **92**:946, 1910.

the number). In this second series of thymectomies they obtained results only slightly different from their previous ones. They again stated their thymectomized dogs died within a year of the operation and showed at necropsy extreme dilatation of the heart but no changes in the skeleton. In this report of their further experiments Nordmann laid special emphasis on the extreme muscular weakness displayed by the thymectomized animals, and went so far as to imply that intussusception occurring in two of these animals about three months after operation might have had a basis in the disturbance of the muscular system occasioned by deprivation of thymus.

This second series of experiments Nordmann performed in whole or in part with the aid of a Meltzer insufflation apparatus[123] and entered the thorax by way of an incision through the bony ribs on the right side instead of on the left.

Although the results obtained by Nordmann and Hart were positive and exceedingly well marked, they differed materially from the results of Matti and from those of Klose, particularly with reference to the skeleton. In his review of the work of Nordmann and Hart, Matti states: ". . . the operative method chosen by Nordmann appears to offer no absolute guarantee for the complete removal of the thymus" [4] (p. 53) and further: "The great majority of authors . . . will agree with me in the conjecture that the method chosen by Nordmann would be the cause, of a considerable number, at least, of incomplete thymectomies and negative results in so far as changes in the bones are concerned." [4] (p. 54). No operative method, however perfect, is any guarantee of complete removal of the thymus, and it is not fair to deny to Nordmann the possibility that his thymectomies were complete because of the use of a lateral instead of a median incision. Indeed, there is reason for thinking that the later thymectomies of Nordmann may have been more uniformly complete than those of Matti, since, though he used an inferior exposure, he had the benefit of time because of his use of intratracheal insufflation, whereas Matti was compelled, once the thorax was opened, to dissect out the thymus with all possible haste in order to avert asphyxia.

No matter how the relative merits of the question just discussed may be settled, the fact remains that Nordmann published new experiments in 1914,[124] on eight litters of puppies in the second week of life,

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123. Meltzer, S. J.: The Method of Respiration by Intratracheal Insufflation, Its Scientific Principle and Its Practical Availability in Medicine and Surgery, Med. Rec. **77**:477, 1910. Die methode der intratrachealen Insufflation, ihr wissenschaftliches Prinzip und ihre praktische Anwendbarkeit, Berl. klin. Wchnschr. **47**:566, 1910.

124. Nordmann, O.: Experimentelles und Klinisches über die Thymusdrüse, Deutsch. med. Wchnschr. **40**:1702, 1914. Experimentelles und Klinisches über die Thymusdrüse, Arch. f. klin. Chir. **106**:172, 1915.

using intratrachial insufflation and also the operative method for removal of the thymus advocated by Matti himself. He fed the puppies on a mixed diet and provided for them complete freedom out of doors. He sacrificed them at varying periods after thymectomy, in order to avert the error of overlooking transitory changes, allowing some of them to live for more than a year. His thymectomized dogs developed no symptoms or pathologic changes of any sort whatsoever in any organ of the body, or in the bones. No thymus rests were found in a single instance, although a particularly careful search appears to have been made by means of serial section of all suspicious bits of tissue.

The negative results obtained by Nordmann in his last series of experiments have great importance, especially in view of the positive results of his previous experiments. In the light of his more recent experiments, he concluded that his former positive findings had been due to other causes than deprivation of thymus — for example, to infection—and that the thymus is not essential to life and its removal in young dogs is meaningless.

It should be added that Nordmann removed the spleen from several animals, in addition to the thymus, but did not discover any pathologic developments, such as Klose described. One female dog deprived of both spleen and thymus bore normal puppies.

Nordmann suggests that the results of the thymectomies obtained by Matti and Klose may have been due to "domestication." He charges Klose with the use of an inbred type of dog, particularly liable to the spontaneous development of rickets.

In 1911 Paton[125] returned to the problem of the relationship of thymic and testicular functions in the guinea-pig. Was it not possible that each organ exercised an independent, stimulating influence on growth rather than an inhibitory influence on the other, as he had previously thought? To put this new hypothesis to the test, he first determined in a series of four guinea-pigs and four control animals that castration before sexual maturity, i. e., according to his method of reckoning, in animals weighing less than 300 gm., did not affect the weight of the animal. He next found, by combined extirpation of both thymus and testes in a series of sixteen guinea-pigs, again weighing less than 300 gm., that retardation in growth estimated in terms of weight did ensue. Since he regarded his previous experimental work as having proved that loss of the thymus alone has no influence on growth, he considered the chain of experimental evidence necessary for the establishment of the new hypothesis complete. His final conception

125. Paton, D. N.: The Thymus and Sexual Organs. Their Relationship to the Growth of the Animal, J. Physiol. **42**:267, 1911.

of the relationship between thymus and sexual organs was, therefore, that each stimulated growth in the immature animal independently of the other and consequently the hypertrophy of the testes, when the thymus was removed, and the persistence of the thymus in an uninvoluted state, when the testes were removed, in sexually immature animals, were in the nature of compensatory phenomena.

On performing similar experiments with female guinea-pigs he was unable to produce changes corresponding to those obtained in the male.

It should be added that in the experiments just described Paton again used control animals from "the same batches" but not from the same litters of animals and that he seems again to have assumed that the excisions of the thymus had been complete.

Lucien and Parisot published the preliminary report[126] of their experiments in 1907 and the complete report[127] in 1910. They extirpated the thymus from five rabbits of a litter of ten, and from four of another litter of eight, with a view to the study of the effects on growth and development of the animal as a whole and of the skeleton and on the various organs, including the spleen and the organs of internal secretion. The animals not operated on served as control animals. All the animals at the time of operation weighed between 303 and 735 gm. (the ages are not given). They used the method of operation of Basch, entering the thorax through a median incision in the upper part of the sternum. They kept their animals under observation for variable periods of from two and one half to fourteen months.

Lucien and Parisot noted that when the thymus was taken out in comparatively young rabbits (weighing between 300 and 400 gm.) the growth in weight of the operated animals underwent considerable retardation, which reached its maximum in from one and one half to two months after operation, but thereafter gradually became less until, finally, the weight of the operated rabbit equalled or even exceeded the weight of the control rabbit. On the other hand, the longer the operation was deferred the less marked was this temporary retardation in weight. The maximal difference between the weights of the pair of rabbits oldest at the time of operation was 230 gm. and occurred three weeks after operation, but between the weights of the pair of rabbits youngest when operated on it amounted to 505 gm. and

126. Lucien, M., and Parisot, J.: Influence de la thymectomie sur la croissance, Mém. Soc. de méd. de Nancy, 1907-1908, p. 259. Also: Rev. méd de l'est **40**:757, 1908. Variations pondérales consécutives à la thymectomie chez le lapin, Compt. rend. Soc. de biol. **65**:261, 1908.

127. Lucien, M., and Parisot, J.: Modifications du poids de la thyroïde après la thymectomie, Compt. rend. Soc. de biol. **66**:406, 1909. Contribution à l'étude des fonctions du thymus, son influence sur la croissance, le développement du squelette et l'évolution des organes, Arch. de méd. expér. et d'anat. path. **22**:98, 1910.

was observed about two months after the thymus had been removed. In the entire series of nine pairs of animals this temporary retardation of growth in weight of the operative animals took place.

When Lucien and Parisot studied the bones of their rabbits they found that those of the thymectomized animals were shorter and weighed less than the corresponding bones of the control animals. The adjacent epiphyses of tibia and femur of one rabbit, i. e., those forming the hind knee joints, showed definite thickening, but, with this single exception, none of the bones of the thymectomized rabbits showed any change either in contour, rigidity or calcium content as determined by chemical analysis. Furthermore, roentgen-ray examinations failed to demonstrate any differences. Histologic examinations of the bones apparently were not made.

Lucien and Parisot[127] seem to have limited their studies of the effects on the viscera of removal of the thymus to a comparison of the weights of certain organs in six pairs of thymectomized and control animals. They found that the weights of the suprarenals, hypophysis, liver, kidneys and spleen in the operated and control animals corresponded. The thyroids of the thymectomized animals, however, weighed slightly less, absolutely, and, in all but one instance, relatively, than the thyroids of the control animals, and the testes of the two male thymectomized animals weighed less than the testes of their control mates. The difference in the weights of the testes was particularly marked in that pair of animals killed the sooner (two and one half months) after thymectomy; the testes of the thymectomized animal were but one-fifth the weight of the testes of the control rabbit. The ovaries of three thymectomized animals were slightly larger than those of the control rabbits and the follicles seemed a little retarded in development. They observed that the spleens of some of their thymectomized animals contained larger and more numerous malpighian bodies than the spleens of the control animals.

Lucien and Parisot concluded that the thymus was not essential to life but produced temporary retardation in the growth of the animal as a whole and of the skeleton, and might exert an influence on the thyroids and genital glands.

Fulci[128] made partial extirpation experiments of the thymus in rabbits, dogs and cats, in order to determine the regenerative capacity of the mammalian thymus. His experiments on rabbits numbered over one hundred. The rabbits were for the most part young animals, not much more than a month old and weighing about 250 gm. when operated on. Some rabbits more than 3 years old were also employed.

128. Fulci, F.: Die Natur der Thymusdrüse nach Untersuchungen über ihre Regenerationsfähigkeit bei den Säugetieren, Deutsch. med. Wchnschr. **39**:1776, 1913.

The dogs used in the experiments were taken from two litters of puppies (one of them consisting of thirteen animals) and were from 14 days to 3 months old when the operations were performed. Two full-grown dogs were also used. The experiments in the cat were carried out on animals more than 1 year old.

Fulci endeavored to remove the greater part of the thymus "at least four-fifths" of the total gland, leaving the remainder in the thorax for subsequent study. It is not clear, however, that he could have known exactly how much thymus he did leave behind in his experiments on rabbits, because of the character of the operation which he performed in that animal. He first made a transverse incision through the soft parts at the base of the neck and by means of blunt dissection forced a passage into the anterior mediastinum to the thymus. He next caught the thymus with forceps and pulled it through the hole which he had formed into the neck, then cut off what he estimated to be four-fifths of the gland and allowed the rest to fall back into the thorax. It is obvious that one half or more of the thymus happening to be separate from the rest or to have become broken off, might have remained concealed in the thorax and its presence remained unsuspected. Fulci's partial thymectomy experiments on dogs and cats were, on the other hand, performed under direct guidance of the eye after a complete exposure of the entire gland had been obtained by means of incision through the sternum. Fulci killed his animals after observation periods of from thirty hours to three months.

We shall not describe in detail the results obtained by Fulci[129] in his experiments. He found that in the majority of his animals the thymus rests showed evidences of regeneration, which seem to have been chiefly histologic, irrespective of the period of time which had elapsed since the operation or the age of the animal at the time of operation. These evidences of regeneration were essentially the same in the three species of animals used. The only exceptions occurred in some of the rabbits which, operated on in the cold of winter, emaciated rapidly, became ill and died as the result of continued exposure. The experiments on these latter Fulci thought demonstrated that the thymus can become completely atrophic in a few days. Fulci's studies of the regenerating thymus rests led him to believe that it was possible to trace the development of the small round cells through several intermediate stages back to the reticular cells of the thymus, which latter are generally admitted to be epithelial elements—in other words, that the small round cells, "the thymus lymphocytes," were derivatives of the

129. Fulci F.: Sulla rigenerazione del timo dei mamifori, Pathologica, Genova **5**:259, 1912. Ricerche sperimentali sui trapianti del timo, Pathologica, Genova **6**:690, 1913.

epithelium. He concluded that the thymus was, as Stöhr[130] maintained, a purely epithelial organ. Fulci admitted, however, that it was impossible to be absolutely certain that the small round cells had not migrated into the thymus from without, as Hammar,[9] Johnson,[131] Maximow[13] and others have held.

In the main, Fulci's partially thymectomized animals showed no disturbances of growth, but some appeared to develop less well than the control animals. One of Fulci's partial thymectomy experiments, however, resulted in such inexplicable changes as to require special mention. Fulci removed 0.28 gm. of the thymus of a rabbit weighing 580 gm. and less than 2 months old at the time of operation. Three months later he killed the animal and found that it then weighed 1,070 gm. and its control 1,250 gm. The thymus rest of the operated rabbit weighed 1.40 gm.; the untouched thymus of the control rabbit weighed 1.77 gm. The animal operated on not only weighed less and was smaller than the control animal, but showed marked deformity of the bones of the extremities, which were thicker and shorter than those of the control animal, and were bent, particularly the femurs. Fulci concluded from this experiment that in the period of development even transitory "hypothymie" can produce severe rachiticlike changes in the skeleton.

Fulci gives some interesting figures showing the marked variations in weight of the thymus in animals of the same litters and of essentially the same weights and also the frequency with which the thymus exists unatrophied even in adult animals. For example, in two rabbits more than 3 years old Fulci found normal thymus glands, each weighing more than 3 gm., and in a dog several years old, weighing 14 kg., he found a thymus weighing 12 gm.

Magnini[132] extirpated the thymus gland from rats. Unfortunately, his work is not available to us in the original so that we are obliged to quote the description of his experiments given by Pappenheimer.[23] "Magnini has also published an article dealing with the effect of thymus extirpation upon white rats. Young rats, less than 60 or 65 gm. in weight, showed effects noticeable within a few days after the operation. There developed a progressive cachexia which terminated fatally after from forty to fifty days. Older rats, up to 70 gm. in weight, survived for about two months, whereas still larger animals recovered and remained alive. By the injection of suitable doses of thymus extract,

130. Stöhr, P.: Ueber die Abstammung der kleinen Thymusrindenzellen, Anat. Hefte **41**:105, 1910.

131. Jonson, A.: Studien über die Thymusinvolution; die akzidentelle Involution bei Hunger, Arch. f. mikr. Anat. **123**:390, 1909.

132. Magnini, M.: Le funzioni del timo ed i rapporti fra timo e milza, Arch. di fisiol. **11**:333, 1912.

it was possible to prevent the ill effects of the thymus extirpation. The spleen was found enlarged in 75 per cent. of the thymectomized rats, but no microscopic changes were observed in liver, suprarenals, hypophysis, or testicles. No mention is made of alterations in the skeleton system" (p. 477).

Pappenheimer,[133] with the aid of an apparatus for the maintenance of positive intrapulmonary pressure, removed the thymus from rats aged three weeks or less, controlling from the same litters. The animals which he used came from twenty-six litters and totalled 118 rats, eighty-two of which were thymectomized. Owing to an epidemic of dysentery, however, his animals became reduced to eleven thymectomized and ten control rats. Ten of the eleven thymectomized rats were proven by means of serial section to be thymus free. The period of observation after operation ranged from thirty-one to 131 days. Pappenheimer found that removal of the thymus did not produce disturbance in growth or development; in fact, he discovered no pathologic changes anywhere, not in any of the organs of internal secretion or in the skeleton. Inasmuch as his experiments are probably the only ones in the higher mammals in which thymus rests were actually proven not to exist, his negative experimental results have more value than the positive results obtained by other investigators, for it is obvious that these latter may have been caused by some other factor than deprivation of thymus.

In a subsequent paper Pappenheimer[134] reported the results of further thymectomy experiments in the rat, in which the complete removal of the gland was again made certain by serial section of the neck, including the thyroids and tissues of the upper half of the thorax. The results of these experiments were also negative.

Pappenheimer's second paper[23] is especially interesting and important, however, for another reason, namely, because it records an "epidemic" of rickets which developed alike among the animals deprived of the thymus, their control animals and the untouched animals from the stock cages. The affected animals showed typical rachitic lesions of the bones and, in addition, alterations of the teeth such as Erdheim[135] described after parathyroidectomy.

Pappenheimer's second series of experiments prove once again that complete removal of the thymus from the rat during the first weeks of life is not necessarily followed by any symptoms or changes in the

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133. Pappenheimer, A. M.: The Effects of Early Extirpation of the Thymus in Albino Rats, J. Exper. M. **19**:319, 1914.

134. Pappenheimer, A. M.: Recent Advances in Thymus Research, Am. Med. **9**:212, 1914. Ueber Thymusausschaltung bei weissen Ratten, Centralbl.-f. allg. Path. u. path. Anat. **25**:249, 1914.

135. Erdheim, J.: Zur Kenntnis der parathyreopriven Dentin-Veränderung, Frankf. Ztschr. f. Path. **7**:238, 1911.

organs, and that the thymus in the rat is not essential to life. Moreover, Pappenheimer's description of the outbreak of rickets among his rats, causing bone lesions identical with those described by Klose and death of the animals in an emaciated state, raises the question whether the results reported by Klose could not have been due to a similar cause and in no way related to deprivation of thymus function. Klose furnishes almost no information concerning his experiments in the rat.[136]

Halnan and Marshall,[137] in 1914, reported the results of their thymus extirpation experiments in guinea-pigs. They found that removal of the thymus in young guinea-pigs, most of which weighed about 150 gm., did not affect growth, and that castration led to an arrested atrophy and subsequent hypertrophy of the thymus, and, more important, as bearing on Paton's work of 1911, that simultaneous removal of the testes and thymus did not affect the growth of immature animals, and that thymectomy was not followed by hypertrophy of the testes. Thus, they failed entirely to confirm the results of Paton in regard to the effect of thymectomy on the immature testis. Their paper concludes with a note from Yule showing by methods of statistical analysis that the differences which Paton found in the testes of thymectomized and control guinea-pigs were not greater than might occur from chance variation. Halnan and Marshall do not seem to have made histologic examinations in order to exclude the presence of thymus rests.

Flesch's experiments[24] (1915) on the rat were published from Rehn's clinic in Frankfurt, from which Klose's work also emanated. As might be anticipated, Flesch's investigations have an intimate bearing on those of Klose, constituting a special inquiry into the latter's theory of a specific interrelationship between thymic and splenic function.

Flesch removed the thymus from more than one hundred rats, twenty-four of which survived and, with their control mates, constituted almost all, if not his entire, series of experimental animals. Fourteen of the thymectomized animals were killed with their control mates, one, two and three weeks after operation; ten died naturally

136. Turning again to Klose's report of his thymus extirpation experiments in the rat in the light of Pappenheimer's experiences, we are struck anew by its vagueness. All those details which the reader of a scientific publication demands as a preliminary to the acceptance of the results stated or the conclusions drawn, are lacking in Klose's article. We cannot find that the author even stated that his control rats remained free from the changes in the bones which he found in the operated animals.

137. Halnan, E. T., and Marshall, F. H. A.: On the Relation Between the Thymus and the Generative Organs and the Influence of These Organs on Growth, Proc. Roy. Soc. Lond. **88**:68, 1914.

from thirty to forty-two days after operation, and two, remaining normal, were killed on the eightieth day. Several additional thymectomized rats were used for studies on the blood. Flesch removed the thymus when his animals were from 4 to 6 weeks old. The fourth week he discovered was less favorable for operation than either the fifth or the sixth week, both because the mortality at that time was higher and because the effects produced were less pronounced. The seventh week proved also less desirable for operation, because the thymus had by that time become adherent. The exact ages of the majority of the animals used do not seem to have been known, but were computed from the weights.

Flesch operated without the aid of any artificial respiration apparatus, using a median incision carried through the sterum.

The rats recovered from the operation rapidly. "They sprang about in as lively a manner" as the control rats and showed no abnormalities of appetite. Indeed, they seem to have exhibited none of the symptoms described by Klose and Matti, except an inconstant retardation of growth[138] and also an inconstant dryness and coarseness of the hair. Indeed, with the exception just stated, they appeared normal until two or three days before they died. In Flesch's words: ". . . after having appeared somewhat less vigorous for two or three days than the control animals, we found them in the morning dead in the cages, frequently partially eaten by the surviving controls."

The blood of the few thymectomized and control animals examined three and one half weeks after operation showed essentially no changes.

At the necropsies Flesch could find no cause for death, and for that reason concluded that death was due to loss of thymus. The spleens of the operated and control animals showed no constant differences; there were no characteristic alterations in weight, but in most cases hypertrophy of the follicles, increase in the megalokaryocytes, proliferation of myeloid tissue and of connective tissue, frequently with terminal sclerotic atrophy of the follicles.

Apparently, Flesch did not examine the bones or organs of internal secretion. It is certain, however, that rickets of such severity as to give rise to gross skeletal deformities, such as Klose described in his thymectomized rats, and which would have been unmistakable even on gross inspection, did not occur.

Flesch concluded that the thymus cannot be extirpated completely from the rat and in almost all of his thymectomized animals he discovered at necropsy a thymus rest, often of microscopic size. It is

138. Between the eleventh and twenty-eighth days after operation, in five out of nine animals; at the time of necropsy, five to seven weeks after operation, in six out of eight animals.

exceedingly interesting and important to note his statement that the presence or absence of thymus rests made no difference in regard to the effect of thymectomy, and that in most cases the thymus rests showed signs of degeneration, though in some cases evidence of regeneration such as was described by Fulci. Of the two animals which it was necessary to kill at the end of eighty days, one had a comparatively large rest, the other had no rest, but the most pronounced changes in the spleen. The rests of thymus actually found by Flesch seem to have been parts derived from the third pharyngeal pouch, since he described them as being the tip ends of the cervical processes.

Nowhere does he mention finding the lobe of thymus derived from the fourth pouch, situated in the substance of the thyroid, to the presence of which Klose ascribed all of his negative results in the rat. Yet we find Flesch using Matti's and also Klose's subterfuge of invoking the presence of that rest to explain results not in agreement with preconceived ideas. For instance, Flesch presupposed the presence of a concealed lobe from the fourth pouch when the rest actually found showed signs of degeneration instead of regeneration[139] or when the spleen failed to show the changes which he apparently thought it should. Flesch displayed several other evidences of the influence of Klose. For example, he endeavored to divide the course in the case of his thymectomized animals into the "latent period" (from one to ten days), "stage of adiposity" (from eleven to twenty-eight days), "stage of cachexia" (from twenty-eight to forty-nine days), although it is perfectly clear from his description that these "stages" did not exist in his experiments. He denied the validity of Klose's main thesis, that the spleen can substitute for the thymus when the latter has been removed, though his statements in one or two places seem to imply the existence in his mind of a belief in such a relationship and he expressed the opinion that the changes in the spleen are not more than a part of the general reaction of the body to loss of the thymus. He concluded that the thymus is essential to life in the rat.

Pappenheimer's investigations effectually disprove the work of Flesch or, at least, the only positive result which Flesch obtained,

139. Flesch's reasoning appears to have been as follows: If the thymus rest found was degenerated, when according to his preconceived ideas it should have been in a state of hyperplasia, a concealed thymus rest must have been present elsewhere which was meeting all the requirements of the body so fully that all other thymus tissue was rendered superfluous. If this concealed rest had been discovered, it would have been found to be in a state of hyperplasia or undergoing an actual regenerative change in contrast to its degenerating or degenerated neighbor. If the only rest which Flesch was able to find was in a degenerated state, it would seem to us necessary to conclude that there was no longer any need for thymus function in that animal, provided, of course, that the blood supply of the rest had remained intact.

namely, that extirpation of the thymus in the rat causes death.[140] Pappenheimer's investigations also disproved Flesch's statement that the thymus cannot be extirpated completely in that animal. The short illness of Flesch's rats ending in death, as well as the slowly progressing fatal illness described by Klose and Magnini, must be explained on some other ground than deprivation of thymus function. Indeed, if Flesch's work be considered without reference to that of Pappenheimer, it appears altogether improbable that deprivation of thymus function had any connection with the death of his animals, since, as Flesch himself states, the outcome was the same whether or not thymus rests were present. The changes which Flesch reported in the spleens of his thymectomized rats have no significance. His entire discussion of the results of his experiments is confused, it would seem, because he continually halts between his own instinctive judgments and the conventional interpretations of others.

Tongu[141] extirpated the thymus gland according to the method of Basch from twenty dogs and fifty rabbits and implanted portions of it into the abdominal cavity, the spleen, the muscles and underneath the skin. He found that the thymus tissue transplanted into the muscles, spleen and beneath the skin disappeared very rapidly, but that the thymus transplanted into the peritoneal cavity might withstand absorption for as long a period as two months. It invariably showed signs of atrophy, however, and never any signs of regeneration. Though the number of his experiments was large, the number of his successful experiments was exceedingly small. The majority of his animals died soon after operation from infection or shock. He regarded his thymectomies as complete. He noted no pathologic changes referable to deprivation of the thymus function in any of his thymectomized animals.

Renton[142] reported a series of thymus extirpation experiments on both guinea-pigs and rabbits. His original aim was to determine the effects of transplantation of the thymus and his extirpations were undertaken with that end in view, namely, to obtain a series of animals showing evidences of thymus insufficiency which would be capable of reacting in a measurable way to thymus transplantation. Accordingly he removed the thymus from ten guinea-pigs (ages not stated), when

140. It will be remembered that eight of Flesch's animals died following extirpation of the thymus but two did not die. One of these latter was found at necropsy to have a large thymus rest and the other extensive changes in the spleen.

141. Tongu, Y.: Recherches expérimentales sur la transplantation du thymus. (Prèmiere étude.) Mitt. a. d. med. Fakult. d. k. Univ. zu Tokyo **14**:259, 1915.

142. Renton, J. M.: An Experimental Study of Extirpation and Transplantation of the Thymus, Glasgow M. J. **86**:14, 1916.

they reached weights of 100 gm. and from six rabbits from 15 to 20 days old. Both sets of animals were controlled, the rabbits apparently from the same litters. He does not indicate the length of time during which his guinea-pigs were kept under observation, but four of the thymectomized rabbits survived and were watched for more than two months after the operation. Neither the thymectomized guinea-pigs nor the rabbits showed any changes in the rate of growth or alterations in the thyroids. Though there was considerable variation in the structure of the thyroid in both operated and control guinea-pigs, there were no variations common to one group and not to the other. In one of the rabbits a thymus rest no larger than a pinhead was found which showed no evidences of regeneration, but in the other three no rests could be discovered. (The thyroids were included in the examination for rests). Renton does not state what measures he took to exclude thymus rests in his thymectomized guinea-pigs, but seems to have thought extirpation in that animal complete. Renton's experiments in both rabbits and guinea-pigs were, therefore, negative.

Renton and Robertson[143] performed thymus extirpations on seventeen dogs, four of which died shortly after the operation, and five not long afterward from disease or accident. The remaining eight dogs formed satsfactory subjects for study. The youngest of the eight animals at the time of operation was 13 days old, the oldest 25 days. Each animal was controlled by one or two dogs from the same litter. They were observed for varying periods of from eighty-seven to 156 days after operation and were then killed. The first animals operated on were confined in a laboratory, but were taken out of doors to exercise each day. In the subsequent experiments the animals were housed on a roof where there was an enclosure forty feet long, which permitted exercise in the open air but was cold and damp. In the latter part of their experiments the animals were taken off the roof and turned loose in a field outside the building for a part of each day. One pair (Experiment No. 2) received little exercise and were the most severely affected; another pair (Experiment No. 1) had considerable exercise, and remained almost normal. The dogs were given a diet of milk, porridge, biscuits and bones.

The operation used was similar to that employed by MacLennon on the rabbit. The sternum and first and second cartilages were bared, the two cartilages on each side ligatured a short distance from the sternum and incised mesial to the ligatures, i. e., between them and the sternum; the sternum was cut across below the second costal cartilage, and the upper part of it, thus isolated, was removed. After the

143. Renton, J. M., and Robertson, Madge E.: Thymusectomy and Its Relationship to Rickets, J. Path. & Bacteriol. **21**:1, 1916.

thymus had been dissected out, the thorax was closed by tying together the loose ends of the ligatures which had been left long for that purpose, thereby bringing the cut ends of the costal cartilages on the two sides in apposition. Renton and Robertson did not use any artificial respiration apparatus and had to contend always with unilateral and sometimes with bilateral pneumothorax.

The changes which occurred in Renton and Robertson's animals can best be indicated in their own words: "The greater number of our animals, as will have been noted, developed definite rachitic changes which we will now describe more fully. The animals remained healthy till they were from 7 to 8 weeks old, when slight listlessness and lack of energy was usually noticed first. By the ninth week definite enlargement of the forejoints was usually present, and the animal began to put its whole foot to the ground instead of the toes. Gradually, the joints enlarged further, the bones began to bend, and the feet assumed the extended and valgus position. When the animals were from 12 to 14 weeks old the rickets appeared to be at its worst, and at this time many of the animals became feeble and walked with difficulty, the coat being rough and in some instances verminous; a certain number died at this period, but others again showed signs of improving strength. This is very similar to the cachetic stage of Klose and Vogt and Matti, although it developed rather earlier and more of the animals recovered. No abnormal or perverted appetite was observed at any stage. These symptoms correspond almost exactly with those described by Basch, Klose and Vogt, and Matti, if one excepts the adipose stage of Klose and Vogt which we never saw, with, however, this great difference, that the changes were not confined to the thymusless animals but were equally marked in the controls.

"From a study of these operations we were unable to convince ourselves that removal of the thymus had any effect on the animals whatsoever. In only one case (No 3) did the thymusless animal show rather more marked rachitic changes than the controls, while in two cases, Nos. 1 and 5, the operated animals remained quite healthy. In all the others the changes were present to about the same extent, and, what is more, commenced to develop at the same time, namely, when the animals were about 8 to 9 weeks old. The age at which the animal was operated on did not appear to make any difference in the time at which rachitic changes were first noted. The fox-terriers (Case 4) did not show rachitic changes until they were 99 days old, and then only slightly, but this must be attributed to their being less susceptible to rickets than some other breeds. It is to be noted that one dog, Case 8, was kept alive for 181 days, and that apart from the rachitic deformities it was perfectly well when it was killed. Further, it is to be noted

that one control was sometimes more affected than the other, showing that there is a certain amount of individual susceptibility to rachitic changes" [143] (p. 5).

Postmortem examination of the bones of the affected animals showed them to be so soft that they cut readily with the scissors, except the bones of one pair, which offered some resistance. The finer changes in the animals more severely affected consisted of marked broadening of the epiphyseal cartilage, irregularity on the diaphyseal side, the presence of irregular masses of osteoid tissue immediately adjacent to the cartilage, "whilst peninsulas and islets of practically unchanged cartilage were observed amongst this tissue." The zone of preliminary calcification was absent and, in the less affected animals, irregular, cyst-like spaces were present in both diaphysis and epiphysis.

Renton and Robertson could find no changes in the thyroids of seven animals operated on which were not present in the control animals. The spleen, liver, pancreas and spinal cord examined in a number of animals showed no changes of note.

In three dogs the authors removed both thymus and testes with no very definite results.

It is particularly to be regretted that Renton and Robertson's descriptions of the pathologic changes in the bones of their dogs are so meager and the illustrative microscopic preparations so poor. The changes enumerated, which are limited to those occurring in the metaphyses, are typical of rickets and an absence of all evidence of calcium deposition in the cartilage, if not due to improper decalcification, could not readily be interpreted otherwise. The only "higher power view" shown, that of the lower end of the radius of dog No. 3, is highly suggestive of rickets.

Renton and Robertson's work appears to us to be extremely important, from the possible light which it throws on the positive experiments of others. It affords an experimental demonstration of the fact that dogs deprived of the thymus gland and under observation for the development of certain changes in the skeleton from that cause may develop exactly those changes from an entirely different and wholly unrelated cause, namely disease.

The investigator who most recently studied the effects of removal of the thymus in the guinea-pig was Park.[144] He removed the gland from eighteen guinea-pigs (which survived intercurrent disease and accident to the end of the experiment) between the first and fifth days of life. Twelve of them were controlled from the same litters and six from different litters but by animals of almost identical age. In

144. Park, E. A.: Extirpation of the Thymus in the Guinea-Pig, J. Exper. M. **25**:129, 1917.

two additional experiments performed by Auchincloss and Park and reported in Park's paper, the guinea-pigs were obtained before term by cesarean section and thymectomized immediately. All the guinea-pigs were observed for periods varying from two and one half months to one year. Absolutely no changes could be detected, either in the growth or development of the thymectomized animals or in any of the organs, including the organs of internal secretion and the bones.

In order to exclude the presence of thymus rests in the operated animals, Park studied in serial section the neck tissue of a series of them and of some normal animals and found in almost all an isolated accessory lobe or accessory lobes of thymus in intimate relation to the parathyroid springing from the third pharyngeal pouch. He believed that except for an error in technic these accessory lobes would have been found in all. In some of the thymectomized animals, accessory lobes in the position normally occupied by the main lobes of the thymus, which had been left when the latter were removed, were also discovered.

Park concluded that in all probability the thymus cannot be extirpated completely from the guinea-pig and, consequently, that the guinea-pig is an absolutely unsuitable subject for thymus extirpation, if it is desired to remove the entire gland. His work makes it appear that the thymus extirpations performed on the guinea-pig by all other investigators were partial and should be so regarded inasmuch as none of them examined their animals to make sure that rests were absent, and that their conflicting results must be considered and interpreted in that light.

SUMMARY

It is doubtful whether an unprejudiced person, who read for the first time the literature on experimental extirpation of the thymus, could obtain from it any clear conception as to what the effects of removal of the thymus actually are; probably he would be at a loss to know whether to regard the thymus as essential to life or as an organ devoid of function, or at least of function demonstrable by extirpation. Unfortunately, the more recent experimental work on thymus function, that which falls within the period designated by Klose "the modern era of thymus investigation," has not brought the problem any nearer solution. Indeed, on the whole, it has been productive of more widely divergent results than the earlier work.

Some of the causes for the confused state of our knowledge concerning the effects of removal of the thymus are plain. For example, it is not difficult to see that a considerable part of the experimental work connected with extirpation of the thymus has been of too inferior a quality to permit the hope that it might reveal the truth unless the

function of the thymus chanced to be of so gross a nature that it could not escape the most inexact methods of analysis. The experiments of the earlier and of more recent investigators, who have depended for the removal of the thymus on the method of operation originally employed by Friedleben, cannot be accepted as valid for the determination of the function of the thymus; for if extirpation performed by that method is ever even approximately complete it cannot possibly be so with any regularity. Moreover, the experiments of others who employed better operative methods are of little value or actually worthless because of the careless manner in which they were conducted, the use of unsuitable experimental subjects, the lack of sufficient control animals, etc. Work of the first order in this field is distinctly limited. A great part of the experimental results reported rest on the authority of the word of the investigator alone.

It is also apparent that a number of investigators have drawn conclusions in regard to the effects of removal of the thymus from experimental data which were wholly inadequate, and have not only increased the number of erroneous conceptions thereby, but have planted them firmly in the literature. Soli, for example, inferring that the thymus exerts a regulatory influence over the growth of bone in very young but not in older animals, as the result of one experiment on a rabbit 1 month old, and one experiment on a rabbit 2 months old, or Sinnhuber, stating that the thymus is nonessential to life from experiments on two dogs, neither of which appeared even to have been necropsied in order to make certain that the extirpation had been complete, are only two of a number of possible illustrations. Experiments so limited that chance variaton or dietetic or environmental influences cannot be excluded as the controlling factors do not permit of any induction concerning thymus function. Few seem to have hesitated to draw hard and fast conclusions because of inadequate facts.

It is also manifest that a number of investigators have placed overvaluation on positive experimental results at the expense of negative experimental results. Those who have done this seem to have started out with the assumption that the thymus is possessed of a function susceptible of demonstration by means of extirpation, and that negative results are, from the very nature of things, meaningless. Accordingly, in forming conclusions they have considered only positive results. It must be clear to everyone, however, that in this particular field of investigation the positive experimental results which have been obtained do not have more significance than the negative results, inasmuch as they have consisted in the development of changes in the thymectomized animals which are not specific but may be brought about certainly in other ways than by removal of the thymus gland, or may develop spontaneously. In this way it has come to pass that a discrepancy has arisen

between the actual results of the experiments themselves and the conclusions drawn from them, which appears not on the surface but becomes apparent after study of the experimental literature. If only the *conclusions* which investigators have drawn from their experiments are considered, as is the case in almost all discussions or presentations of the thymus problem in the literature, a false impression as to the actual experimental results is obtained.

The causes just enumerated in explanation of the confusion of ideas which exists concerning the effects of thymus extirpation cannot, however, be regarded as having been more than contributory. Undoubtedly the fundamental cause lies in the difficulties inherent in the problem itself. The diverse character of the results from the same procedure, namely, thymus extirpation, is sufficient proof either that the elicitation of evidence of thymus function is exceedingly difficult, or else that the thymus is possessed of no function or of no function which is demonstrable by extirpation.

The only certain knowledge in regard to the effects of removal of the thymus which experimentation up to the present time has afforded is surprisingly small, and may be stated as follows: The thymus is not essential to life in the frog, and its complete removal from that animal causes no alteration in bodily growth or development.[145] Similarly, the function of the thymus is not necessary to life in the rat, and its destruction in that animal produces no demonstrable alteration in growth or development or any changes of a morphologic nature in any of the organs, as judged by the ordinary methods of histologic study.[146] Few of the positive results of thymectomy have been actually proved to be false. It is certain only that the remarkable train of symptoms endng in death which Abelous and Billard, and later Camia, announced they obtained in the frog; and those which Magnini, Klose and Flesch variously reported in the rat, must have been due to other causes than deprivation of thymus function.

The reported results of thymectomy fall roughly into three groups. In one, removal of the thymus has been found to be without any effect; in another, removal has been followed by transitory disturbances in health and development; in the third, removal has brought about death with or without changes in growth and nutrition, and alterations in the skeleton and in certain of the organs, particularly those of internal secretion. A considerable number of observers, relatively speaking, have been unable to detect any change, or at least, any change of sig-

145. The statement is based on the work of Hammar,[48] who proved by means of serial sections that thymectomy was complete.

146. The statement is based on the work of Pappenheimer,[18] who also demonstrated that thymectomy was complete by means of serial sections of the tissues of thorax and neck.

nificance, following thymectomy. Comparatively few, on the other hand, have discovered that removal of the thymus produced death, and not a single one[147]—if those investigators previously mentioned whose experimental results have been disproved are eliminated—has found that thymectomy caused death invariably, or, indeed, even in the majority of animals. Almost all those who have reported positive results following extirpation of the thymus, have obtained negative results as well, the two kinds being intermingled in varying proportions. Though the changes reported by some have been limited to single pathologic manifestations or to alterations in single organs and have seemed to indicate that thymus activity was distinctly circumscribed, the changes reported by others, in particular by several of the more recent investigators, have been in the nature of complex diseased states in which the whole animal was affected, and have seemed to imply that the function of the thymus was intimately concerned with the most fundamental of the bodily functions. The positive results obtained by some investigators seem to have little connection with the positive results obtained by others, and in a few instances are even contradictory. Those obtained by some, however, have a certain amount of similarity in that they center about changes in the growth and development of the skeleton (overgrowth of the animal as a whole and therefore increased growth of the skeleton; retardation in growth of the skeleton; retardation in growth in length with thickening of the bones of the extremities; thickening of the latter with bowing; spontaneous fractures; reduced calcium content of the bones; pseudorachitic deformities; osteoporosis; osteomalacia; rickets). At least nine investigators have described in one or more thymectomized animals retardation in growth with rachitic-like deformities, especially in the long bones of the leg. Only two of them (Matti and Klose) have given proof, however, that the pathologic condition in the bones of their experimental animals was in reality rickets. One investigator has shown on fairly good evidence that both the thymectomized and control animals of his experiments alike were affected with rickets.

Several observers have reported alterations in the hair of their thymectomized animals and several have described a voracity of appetite exhibited by the thymectomized animal in contrast to his control mate.

A surprisingly small number of investigators have examined their material for alterations in the endocrine glands. Five have reported changes in the nature of hyperplasia in the thyroid, and two reported

147. We do not know what percentage of thymectomized dogs died in Klose's experiments, but are led to believe from one of his statements that a considerable proportion of them remained alive.

hypertrophy of the medulla of the suprarenal, but not one has reported pathologic developments which are of a convincing character in any of the other organs of internal secretion. Few seem to have studied the organs of internal secretion with especial care and no one has applied to the study some of the more recently developed histologic methods, or made examinations in the period which immediately followed thymectomy.

OUR OWN EXPERIMENTS

THE PLAN, METHODS AND CONDUCT

As already stated, the experiments, which are presently to be described, were undertaken in the belief that it was possible to produce in animals a rickets almost, if not quite, identical with human rickets by means of removal of the thymus gland. Since Matti had used dogs in his experiments and Klose and Basch had reported that the dog was especially prone to develop alterations in the skeleton after thymectomy, we chose that animal for our experiments. Our study centers on the effects of removal of the thymus gland on the skeleton, particularly with reference to the development of rickets, but it actually has a more general application, inasmuch as observations were made on living thymectomized animals and the organs were examined after death, in particular those of internal secretion.

The operation which we employed may be termed the standard for removal of the thymus from animals in which the gland lies in part or almost entirely in the thorax. It was the Basch operation with the minor modification used by Klose, Matti and others, namely, that after the thorax had been opened the thymus was dissected out from below upward instead of in the reverse direction.

The steps of the operation were as follows: The animal was fastened on his back to a suitable operating table and the neck and chest were shaved and cleansed with soap and water. He was anesthetized with ether. A small soft rubber catheter, the end of which was cut obliquely to facilitate passage, was introduced into the trachea to the point at which it was estimated the bifurcation lay. The catheter was connected with a Meltzer intratracheal insufflation apparatus, and the anesthesia was thereafter conducted by means of it. The skin was cleansed with alcohol followed by ether and thereafter painted with tincture of iodin. A square of gauze of single thickness, freshly soaked in collodion, was laid over the prepared area. When it had adhered, sterile towels were spread over the animal and made fast with sterile safety pins in such a way as to leave only the proposed line of incision exposed. The incision was made through the collodion gauze from a point in the neck at the level of the thyroid cartilage, or above, caudalward in the median line to, or beyond, the lower third of the sternum. The edges of the wound were retracted and the front of the bony thorax exposed. The attempt was then made—which was not always successful—to divide the sternum in the median line with a sharp knife from the upper end, which always stood out prominently two thirds of the way or more to the ensiform. The divided portions of the sternum, together with the soft tissues, were pulled apart and the thymus, the lung margins and the beating heart exposed. The pleura was then ruptured—if rupture had not already taken place—and the lower part of the thymus was caught with mouse toothed forceps; its dissection from its thoracic attachments with the aid of a blunt dissector was then begun.

Its separation from the fat covering the pericardium was not difficult because of a distinct line of cleavage which developed as soon as the thymus was pulled upward; its separation was further facilitated by the fact that the thymus in the living animal, in contrast to the fat, has a fairly distinct gray or pinkish color. The separation of the thymus from the pericardium was easy but from the region of the left innominate vein attempt at separation was attended with considerable difficulty because branches from the vein entered the thymus and prolongations of thymus tissue were frequently found to pass behind the vein. These might easily escape observation and be left in the animal. Bleeding was frequently encountered from the small branches of the left innominate vein just referred to. Great care was exercised to avoid injury to the phrenic nerves and to render them free from minute fragments of thymus tissue which tended to adhere to them. In the later experiments it was found possible to separate the thymus from the internal mammary vessels without disturbing the latter. In by far the greater number of experiments, however, the vessels were either tied or, after preliminary stripping to facilitate clotting, were snapped across above the thymus close to the cartilages. Bleeding from the main stem of the internal mammary arteries was never encountered (Figs. 5 and 6).

As the neck was reached, great care was taken to see that a cervical process of thymus was not accidentally torn off from the rest and left in the neck. Usually, as the thymus was curled back on itself from below upward, in the course of the blunt dissection, it could plainly be seen to end on one or on both sides in a rather short, frequently blunt and well defined process. In a number of animals, however, it was found to continue upward on one side, or, rarely, on both, along the great vessels of the neck, in the form of a thin strand which in some instances dwindled, as it ascended, to a thin filament. When this latter was present, it was frequently excised with a knife or scissors; if it became lost in a fat mass, the attempt was made to remove the latter in its entirety. In our earlier experiments the neck was not examined farther than the thymus was found to extend but in our later experiments it was in each instance dissected upward until thyroids and parathyroids on both sides were exposed in the hope of finding isolated cervical lobes of thymus. As soon as the thymus had been removed, the region which it had occupied was searched for thymus fragments with the usual result that numerous small masses of tissue were removed, which proved on subsequent section to be fat and lymph nodes. Thymus tissue was rarely discovered.

While the lungs were kept in an inflated condition, the wound was closed with silk sutures and the gauze dressing held in place by a piece of unbleached muslin provided with holes to admit the passage of the animal's fore legs, and at the sides with tapes which were firmly tied together over the animal's back, thus giving firm support to the injured thorax. The actual operation could be done in ten minutes but usually required, as carried out by us, one half hour or more. The dissection of the thymus out of thorax and neck required only two to three minutes. Most of the time was consumed in closing the operation wound, particularly that in the bony thorax, and in the search for thymus fragments after the gland had been removed. The resulting mortality became less in proportion as we reduced the length of our incision in the thorax and the time spent in searching for possible thymus rests left behind.

As the result of our experience with the operation for removal of the thymus in the dog we obtained the impression, which coincides with Klose's statement, that the thymus gland can be dissected out of the thorax more easily in young than in older puppies, i. e., we found it to be less firmly adherent in the former. In the few thymectomies which we performed in puppies older than 5 weeks, however, we saw no evidence to substantiate Klose's claim that the removal of the thymus is "surgically and anatomically" impossible in puppies beyond that age, and should doubt whether any evidence in support of that view existed. We became convinced that older puppies withstood the operation

better than very young puppies, contrary to Klose's assertion. We also found that it was always easier to remove a large thymus than a small one, particularly one in which accidental involution had taken place.

We removed the thymus in the manner described from seventy-five puppies, the youngest of which was 9 days old at the time of operation and the oldest 7 weeks old. With the exception of five, all the puppies operated on were less than 21 days old at the time of operation. A large number were lost as a result of the operation, the majority dying on the third or fourth day. These latter appeared fairly active and vigorous on the day following the operation, but on the succeeding day they began to look sick and to show labored respiration. In most instances death occurred on the third day. At necropsy the chest was always found filled with a yellow serofibrinous fluid and the lungs were compressed. For some time we were under the impression that our technic must be at fault and redoubled our precautions to avoid infection. We found, however, that the infection rarely occurred when we reduced the length of the incision and curtailed the time spent in examining the thorax for stray fragments of thymus. The operation was not the sole cause of the mortality among our puppies during the first few days after operation. We lost some animals from being trampled on or overlain by the mother while in their weakened condition and also from inanition. In the second and third weeks following operation, several thymectomized puppies which apparently had recovered from the effects of operation, died as the result of distemper in epidemic form, diarrheal conditions, bites from other animals and one puppy died from a localized empyema. The dogs suitable for study thus became reduced to twenty-four thymectomized dogs, three incompletely and twenty-one completely thymectomized, and nineteen control dogs from fourteen different litters. These were observed for periods varying approximately from one month to one and one quarter years after operation. Twelve of the thymectomized dogs were observed for more than seven months, nine for more than ten months and five for one year or more.

The dogs obtained for our experiments were none of them of thoroughbred stock although some had a strong preponderance of one breed or another. The thymectomized animals were controlled, in every instance, from the same litters, and in all but three of the fourteen litters each thymectomized dog had his own control animal. In two of the three litters in which the number of thymectomized and control dogs was unequal there was one control each for two thymectomized animals, and in the third litter there were two control dogs for five thymectomized animals. In most of the experiments the larger, but in some the smaller puppies were selected for operation.

For several hours after operation the puppies were kept away from their mother to avoid injury from trampling or overlying. While suckling and for a short time afterward, thymectomized and control puppies were placed in a medium sized room in one of the laboratory buildings, with the exception of those puppies of our last four experiments; these were kept for us from the beginning by private families. Shortly after weaning was complete, the animals of all the experiments, except the four just mentioned, were sent to the country. The animals of our earlier experiments were sent to the Rockefeller Institute farm, where they were placed in two out of door enclosures measuring about 20 by 15 feet. Later they were transferred to a farm in the vicinity of Baltimore. The others were sent from the laboratory room directly to the latter place. The arrangements at the farm for housing the animals consisted of four kennels opening into wire netting enclosures about 20 feet long and 10 feet wide (Fig. 7). In these enclosures the animals were in the open air continually and obtained considerable exercise. Conditions at the farm were far from satisfactory, however, because there was but poor protection from the cold, which seriously interfered with the health and development of the younger animals, and the dogs came into such close contact with each other that mange, distemper, etc., developing in one animal, were immediately trans-

ferred to all others. Moreover, the soil in the enclosures became so impregnated with ascaris ova that a vicious circle of reinfection became established from which death resulted in some instances. It was with the idea of avoiding these complications that we placed the litters of our last four experiments with two private families in the hope that there the animals would be reared under natural conditions. At one of the two homes, however, in which two of the four litters were quartered, conditions proved to be more unsatisfactory than at the farm, as will be explained later.

The feeding of the dogs was conducted as follows: As soon as weaned they were given bread and milk and later supplied with a specially prepared food made by baking a mixture of corn meal and meat.

The dogs quartered at the Rockefeller Institute Farm were seen by us at irregular intervals of from twenty days to one and two-thirds months, but were under the continuous observation of the manager, who sent us written reports. The dogs at the farm in the vicinity of Baltimore were visited by us at intervals of from one week to one month; those litters of dogs "boarded out" at private houses were seen somewhat irregularly.

The weights of the dogs were taken on the dates mentioned in the protocols and in the more important experiments of long duration form fairly complete records.

Some of the dogs of our experiments we sacrificed at the end of long periods when the development of a pathologic condition dependent on loss of thymus could no longer be expected; others were killed when moribund or in the early stages of distemper, or they died natural deaths, which occurred from a variety of causes. We endeavored to perform the necropsies immediately after death but were not always able to do so—for example, when death occurred at the farm, which is some distance from the laboratory. The necropsies were, in almost all instances, complete and the weights of the organs were taken. Particular care was exercised to make certain that no rests of thymus tissue were present in the animals in which thymectomy had been performed. The region of the thorax in which the thymus normally lies was carefully searched for thymus rests, particularly in the neighborhood of the left innominate vein, and all tissues which bore any resemblance to thymus were removed and saved for examination. The neck was dissected upward on both sides until both thyroids were exposed and all tissue which it was thought might possibly be thymus or contain thymus was preserved. In the smaller animals, particularly in those extremely malnourished, it was possible to remove from the thorax and neck every vestige of light colored tissue, and, in some of the very small dogs, the left innominate vein and great vessels of the neck, together with all adjacent tissues, were dissected out in toto. The denudation of tissues occupying the normal site of the thymus was so complete in some of these smaller animals that serial section study of the resulting mass had almost the same importance as the examination of serial sections of the entire neck. When the adipose tissue was abundant, particularly in large well nourished animals, it was manifestly impossible to section serially all the fat, so that in such cases only those bits of tissue were selected which bore some definite resemblance to thymus tissue. At the necropsies of the thymectomized animals of our earlier experiments we hardened the pieces of tissue saved for serial section separately, but in the later ones wrapped them together in gauze as soon as obtained so that when hardened they formed a single mass.

The tissues were preserved in 10 per cent. liquor formaldehydi in each instance, but in some of the earlier and the later experiments in formol-Zenker fixative. The bones were decalcified in liquor formaldehydi-formic acid mixture according to the technic recommended by Schmorl,[148] and stained

148. Schmorl, G.: Die pathologische Anatomie der rachitischen Knochenerkrankung mit besonderer Berüchsichtigung ihrer Histologie und Pathogenese, Ergebn. d. inn. Med. u. Kinderh. **4**:403, 1909.

with hematoxylin and eosin. Some of the material, about which the question of an early rickets was raised, was decalcified as well in Müller's fluid. We did not use other stains than hematoxylin and eosin for the bones because there seemed to be no indication for so doing. Sections from the viscera, including the organs of internal secretion, were stained with hematoxylin and eosin. The suprarenals of the first eight litters were treated also with sudan III, to obtain some rough idea of the relative lipoid content.

The tissues collected from the thymectomized dogs for examination for thymus rests were cut in serial sections of about 15 to 20 microns and stained with hematoxylin and eosin. In almost all instances, every fourth section was examined with a Leitz objective No. 3 and mechanical stage but in the cases of some dogs, in which the blocks of tissue and consequently the sections, were especially large, the latter were first studied with a dissecting microscope and likely areas picked out and reexamined with a high power objective. The tissues saved for examination for thymus rests from the thymectomized dogs of litters Nos. 12 and 14 were not examined serially, but only by means of single sections taken at different planes through the block, and in the cases of dogs Nos. 7 (Exp. No. 3) and 39[149] (Exp. No. 8), the tissues were not examined at all.

As the result of our examinations of the thymectomized dogs for thymus rests, we found in the thorax of dog No. 5, at a point just above the innominate vein, a mass of normal appearing thymus about the size of a pea and some tissue which we thought was atrophied thymus in the thorax of dog No. 30[150] (see protocol of Exp. No. 6). We could find no tissue remotely resembling thymus in any of our other thymectomized animals. It was not surprising that no rest of thymus was found in the partially thymectomized dogs Nos. 37 and 38 (see protocols Exp. No. 7) because the fragments left at operation were so minute as to make absorption highly probable, particularly if they had been deprived of their blood supply.

THE IDENTIFICATION OF THYMUS RESTS

The identification of thymus tissue in the dog may be exceedingly difficult. Rests of normal thymus often can be recognized with the naked eye in sections stained with hematoxylin and eosin because of the exceedingly deep blue color assumed by the cortex. On microscopic examination, the lobular formation with division into cortex and medulla, and the well known peculiarities of structure and composition of each, readily distinguish thymus from all other tissues. If Hassall's corpuscles are present, they afford certain indication that the tissue is thymus. Only when the section traverses the cortex alone can there be any doubt concerning the identity of normal thymus

149. In the case of dog No. 39, the tissues saved were lost; in the case of dog No. 7, such masses of fat were present—the animal was huge—that serial section study seemed futile and was not attempted.

150. In the case of dog No. 30 we were obliged to depend on single sections for our diagnosis. If serial sections had been available, we think that the diagnosis would not have remained doubtful.

tissue; and if a series of sections exists, such doubt is dissipated by examination of succeeding sections. Atrophied thymus tissue, on the other hand, may readily be mistaken for other tissues and overlooked. The characteristics of atrophied thymus tissue in the dog are as follows: The lobules are usually longer than they are wide and, roughly speaking, have an oval, triangular or diamond shape, or appear as strands, which often are curved or bent and sometimes have a wavy outline. If several lobules are present, they are apt to lie more or less parallel, and are separated widely by fat and connective tissue containing blood vessels. The division of the lobule into cortex and medulla has entirely disappeared, or else the cortex has become reduced to a thin border or line composed of densely packed small round cells, many of which are pyknotic. This border stains a deeper blue than the rest of the lobule. The lobule contains many tortuous and branching blood vessels—some containing blood, and some having thickened walls—small round cells having the appearance of lymphocytes, and, intermingled with the small round cells, the large reticular cells of the gland. These latter have a cell body that stains pink, and a pale blue nucleus.[151] Hassall's corpuscles may also be found presenting their ordinary appearances, but in our experience usually are not present in the atrophied thymus of the dog. Not infrequently reticular cells can be seen in such proximity to each other that doubt is felt whether to regard them as Hassall's bodies or not. Occasionally, help in the identification of thymus is obtained by the discovery of a cyst having high or ciliated epithelium in, or adjacent to, the tissue in question. Sometimes, tissue may be thought to be thymus, even though not characteristic, on account of its situation in the thyroid, or its intimate relationship to a parathyroid.

Thymus tissue is most apt to be confounded with lymphoid tissue, not in the form of lymph nodes which with their follicles, lymph spaces, etc., have quite as distinct a structure as the thymus itself, but with aberrant or atrophied lymphoid tissue. Atrophied thymus may also be confounded with embryonal fat in young and malnourished dogs (the so-called organ of internal secretion described by Pende.[152] When doubt exists whether or not tissue in a given section is thymus, its identity usually becomes clear on examination of succeeding sections in the series; it is in single sections that the diagnosis becomes difficult or impossible.

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151. Various other cellular elements derived from the blood or the fixed tissues of the thymus are also present. We have not observed large numbers of eosinophilic cells in the atrophied thymus of the dog.

152. Pende, N.: Ueber eine neue Drüse mit innerer Sekretion (Glandula insularis cervicalis), Arch. f. mikr. Anat., Bonn **86**:193, Part 1, 1914.

THE QUESTION OF PROOF THAT THE THYMUS GLAND CAN BE EXTIRPATED COMPLETELY IN THE DOG

Since atrophied thymus tissue may present the deviations from the normal just mentioned, the question naturally arises whether its identification is always possible. As already stated, in one of our thymectomized dogs (No. 30) we were uncertain whether tissue seen in the stained sections was thymus or not, but probably would not have remained in doubt had it not been necessary to rely on single instead of serial sections. In dog No. 5 the rest found in the thorax had all the appearances of the normal gland. The only evidence which we possess in regard to the power of thymus tissue to retain its distinguishing characteristics, namely, that derived from the examination of the thymus glands of our control animals and some additional dogs, would indicate that the thymus remains recognizable in dogs within the age limits of our experiments; at least the tissue taken as thymus from this group of intact animals never failed to show indubitable evidences of being such on the most casual microscopic examination and exhibited surprisingly few signs of involution in those animals, whether young or old, which were well nourished. We are prevented, however, from concluding definitely that the thymus in dogs less than 16 months old is, under all circumstances, recognizable on account of our ignorance in regard to the behavior or fate of small isolated bodies of thymus, in particular the accessory lobes in the thyroids or in the neck, or the thin cervical processes of the gland. It seems possible that in the normal animal the cervical elements, exposed as they are to different environmental influences, especially in regard to blood supply, and perhaps being possessed of an inherent tendency toward involution, might atrophy with great rapidity and conceivably vanish altogether, while the thoracic thymus remained essentially unchanged.

WHETHER THYMUS TISSUE CAN ALWAYS BE IDENTIFIED IN SERIAL SECTION

The examination for thymus rests by the serial section method can never prove the thymectomy to have been complete in the case of the dog as in that of the smaller animals (such as the rat, frog and guinea-pig), for the simple reason that the thymus region in the dog is so large that only selected tissues from it can be studied. Complete serial section of the soft tissues of the neck and anterior mediastinum is manifestly impracticable in that animal. Failure to discover thymus rests by means of serial section of suspected tissues at most serves to make complete removal of the thymus probable. If, however, neck and thorax are literally denuded of all tissue in the normal thymus areas and the resulting mass is studied in serial section for thymus

rests with negative outcome, as in some of our smaller malnourished animals, the result approximates in point of validity the similar result obtained in the smaller animals after complete serial study of the entire thymus region. In general, the smaller the dog and the freer the thorax from fat, the more complete the denudation can be and the more valuable become the negative results of serial examination. A small breed of dog is, therefore, much the best experimental subject. If the dog is large (dog No. 7, Exp. No. 3), and fat is abundant, it is very difficult to make certain that even gross rests of thymus do not escape observation.

THE POSSIBILITY AND NECESSITY OF COMPLETE REMOVAL OF THE THYMUS IN THE DOG

Since, then, the complete extirpation of the thymus cannot be proved in the dog, the question very naturally follows whether removal of the thymus in its entirety is possible in that animal. We are almost certain that the thymus can always be extirpated completely from the thorax, but we know that it is impossible always to remove it from the neck. In the first place, if the minute lobes of thymus situated in the thyroids (those derived from the fourth pouch) happen to be present, they cannot possibly be detected or removed at operation, and if the small detached masses of thymus chance to be in the neck, it is not improbable that they would be overlooked and left, especially if situated at some distance from the main body of the gland. Moreover, in the course of dissections of the thymus in young dogs, we ourselves have several times seen the cervical processes dwindle to such filaments as to make the possibility of their complete removal at operation extremely doubtful. In a dog which chanced to come under our observation a filamentous cervical process of the thymus was traced into a fat mass and supposed to end. Microscopic examination of the fat beyond the apparent termination, however, revealed a threadlike core of thymus completely enveloped in fat. Whether or not the thymus can be entirely removed in a given dog is largely, therefore, a matter of chance, dependent on anatomic conditions in the neck, and the question of the complete removal of the gland in the dog resolves itself into the question of the frequency with which thymus tissue concealed from the naked eye occurs in that species. We do not know how common thymus rests in the thyroid glands of the dog are, but we believe that their occurrence in these glands is the exception, not the rule. We are ignorant also in regard to the frequency with which accessory lobes of thymus outside the thyroids occur in the neck of that animal, but have obtained the impression from our own work that they are usually absent.

In regard to the frequency with which ineradicable cervical processes of the thymus are encountered we can form no idea. All that we can say in this connection is that in our experience the cervical processes are short and terminate in blunt, clearly demarcated endings, permitting complete removal, in a large proportion of dogs. Even when the cervical process is long, we have thought that usually it was possible with care to extirpate the thymus completely in the dog, and so far as our own experience teaches, it is possible to accomplish complete extirpation in the majority of dogs. It is impossible, however, to draw an absolutely positive conclusion to this effect.

As a matter of fact, the question whether or not the thymus of the dog can be completely removed, has probably only an academic interest; for if the gland has a fundamentally important function, if, for example, as Klose and Matti think, death and more extensive changes in the viscera are caused by its removal than by the removal of any organ of internal secretion, it is absurd to suppose that it must be extirpated to the last cell in order that signs of insufficiency become manifest. Certainly for all the established organs of internal secretion there is an irreducible minimum and that irreducible minimum is a visible, tangible, measurable portion of the gland. Whether the thymus in the dog can be extirpated entirely may be open to question, but there can be no doubt that in all dogs it is possible to reduce it to a most minute rest, which could scarcely be conceived of as alone being able to sustain the function formerly borne by the gland as a whole.

REGENERATION AND HYPERTROPHY OF THE THYMUS REST

Klose, who is more insistent than any other investigator on the necessity of removing the thymus to the last cell in experiments for the determination of thymus function, has given experimental proof that it is not necessary to eradicate the organ completely in order to elicit typical phenomena of insufficiency (see his experiments on the goat and pig[116]). The apparent inconsistency in his attitude on this subject is amply explained, however, when it is realized that he believes the thymus possessed of a capacity for regeneration not approximated by any other tissue in the body, certainly not by any of the highly specialized tissues, which prevents the fragment left at operation from remaining below the irreducible minimum and the symptoms of insufficiency from continuing for more than a brief period. Klose writes: "It can be said with certainty that partial extirpation of the thymus does not have the slightest significance in experimental animals, for the organ possesses an extraordinary vitality which enables it completely to regenerate in both an anatomical and a functional

sence in a comparatively short time from the most minute rests." [153] [In connection with this statement of Klose's read the accounts of his experiments on the goat and pig.] Aschoff[8] likewise holds that the thymus is vested with this extraordinary regenerative power. Matti[154] refers to the "intense regenerative capacity of the thymus" [4] (p. 60) and again to the "well known intense regenerative capacity of the thymus rests left behind at operation" [103] (p. 804). The credit for the discovery that the thymus regenerates is assigned by the more recent writers to Friedleben.[31] As a matter of fact, Friedleben did record his surprise at the large size and cellular appearance of the thymus rest found at necropsy in one of his partially thymectomized dogs and in one goat, presumably because of the absence of those features in the thymus rests of the other partially thymectomized animals, and suggested the possibility that the rest in the dog might have undergone regeneration. Basch[64, 65] noted in several thymectomized dogs at necropsy rests of thymus which he believed must have regenerated. Flesch[24] found that rests inadvertently left at operation in the rat showed signs of hypertrophy in some animals and atrophy in others. Hammar[46] failed to observe any evidence of hypertrophy of thymus rests in the frog, and in some frogs found that the rests actually underwent involutional atrophy. Pappenheimer[133] saw no sign of hyperplasia of the thymus rest in the rat, and Renton[142] none in the rabbit (observation on a single animal).

Fulci[128] is the only investigator who has made a systematic study of the regeneration of the thymus rest.[155] In one partially thymectomized rabbit that author described an hypertrophy of the rest left at operation approximating a return to the size of the normal gland, but in his other animals, rabbits, cats and dogs, changes of a purely histologic character which were similar to those Jonson[131] reported in the thymus of dogs recovering from starvation. The histologic evidence favoring regeneration, brought forward by Fulci, is extremely strong, and we regret that it is impossible to present it in detail. One observation which he made is, however, so out of harmony with his interpretation of the significance of the changes in the rests that we cannot but

153. Man kann nur mit Sicherheit sagen, die partielle Thymus exstirpation hat bei den Versuchstieren nicht die geringsten, eindeutigen Ausfallserscheinungen im Gefolge, da dem Organ eine ausserordentliche Vitalität innewohnt, die es befähigt, sich von kleinsten Resten aus in kurzer Zeit vollkommen anatomisch und funktionell zu regenerieren[39] (p. 43).

154. It seems probable that Matti's opinion is not derived from his own personal experience, inasmuch as he failed to find thymus rests in the thymectomized animals of his recorded experiments.

155. Fulci, F.: Die Restitutionsfähigkeit des Thymus der Säugetiere nach der Schwangerschaft, Centralbl. f. allg. Path. u. path. Anat. **24**:968, 1913. Fulci also studied the regeneration of the thymus after pregnancy.

wonder if the latter were truly regenerative in nature, namely, that in the majority of instances the histologic alterations in the rest occurred in the same way and were the same in character irrespective of the age of the animal. Two of his dogs and all of his cats were 1 year or more old, and some of his rabbits were 3 years old, when partially thymectomized, and should have altogether outgrown dependence on thymus function. Fulci found that the regenerative process reached completion, on the average, at the end of six weeks. Mention should be made of the fact that in some greatly malnourished rabbits in Fulci's experiments the thymus rest was found atrophied.

So far as we are aware, with the single exception of Fulci, all those who have reported regenerative changes in the thymus rest after operation have done so as the result of impressions.[156] The thymus rest at necropsy was large when none was supposed to have been left at operation, or only a fragment! The conclusion naturally followed that it had regenerated. It is obvious that proof of regeneration based on the comparison of the size of the piece of gland actually found at necropsy with that which the operator thinks he might possibly have left some months or weeks previously, is subject to the gravest error. Perhaps a large piece of the gland was left, when it was confidently believed none remained, or at most a mere particle! Unless the rest was deliberately left at operation under the direct guidance of the eye and its location and measurements recorded, subsequent inferences mean little. Another reason why the conclusions which have been made in regard to the hypertrophy of the thymus rest have but little value is because the possibility that normal growth may have taken place has not been considered. The duration of the period of growth of the thymus in the dog is unknown, Klose to the contrary notwithstanding! Perhaps the "hypertrophy" of thymus rests, which has been reported, means no more than that normal growth has taken place.

We should like to see Fulci's findings corroborated before accepting them as final proof that the thymus regenerates. The isolated examples of hypertrophied thymus rests reported by other investigators we cannot regard as more than suggestive observations.

Our own work does not permit us to express a positive opinion whether or not the thymus rest regenerates. The rest found in dog No. 5 resembled normal thymus tissue and showed no evidence of hyperplasia but there was no means of determining whether it had remained the same size as when left at operation, or had grown from a small group of cells. The small size of the rest in that dog killed

156. Klose does not state the evidence on which he based his remarkable statement with regard to the regeneration of thymus rests. Jonson and Fulci interpreted the changes differently, Jonson as proving the small round cells were lymphocytes, Fulci that they were derivatives of the epithelium.

Fig. 16.—Photograph of a 3 months old dog, fed on a normal diet but confined in a cage from the time when weaning was complete. At necropsy the thymus weighed approximately 3 gm. The bones showed well marked osteoporosis but no sign of rickets. Note bowing of forelegs and enlargements at the joints, such as frequently have been described in thymectomized animals and regarded as the specific effects of deprivation of thymus function.

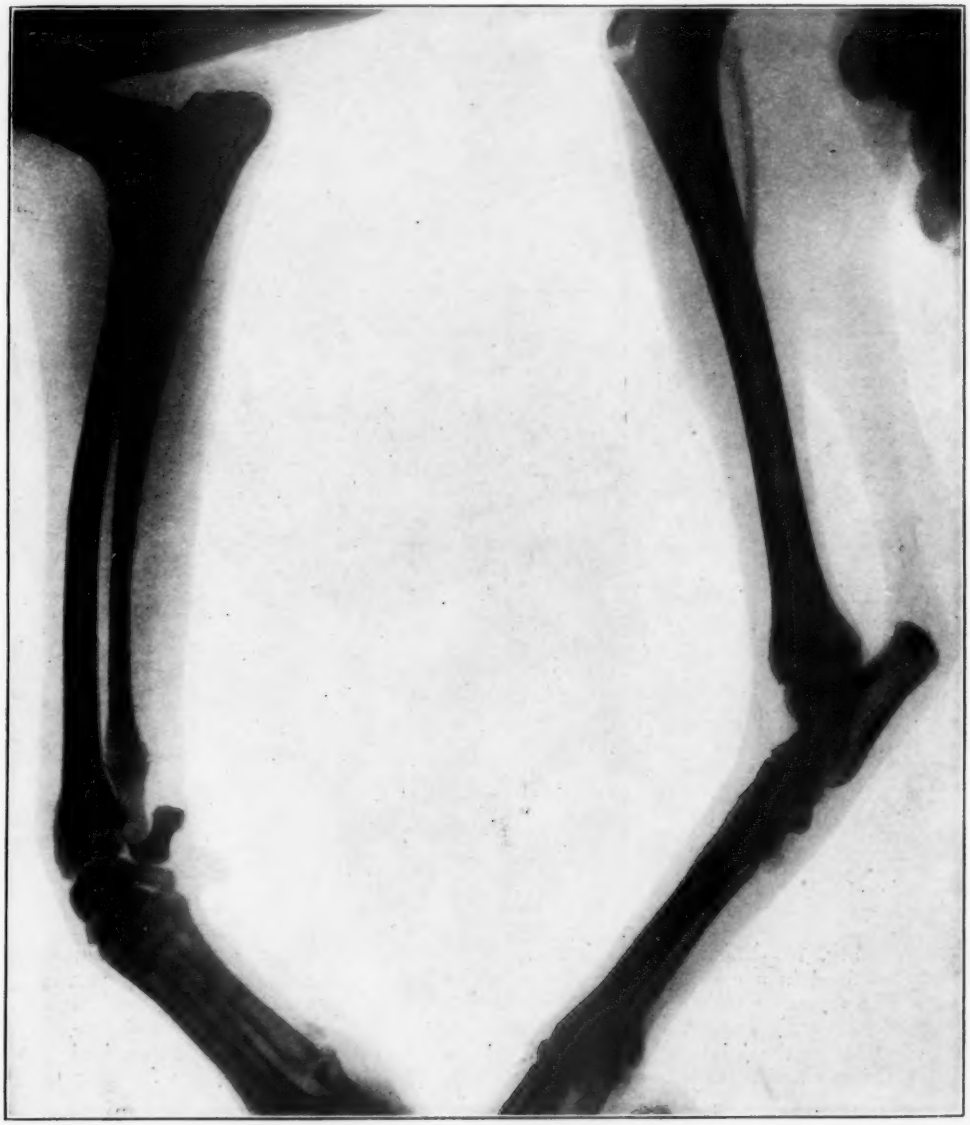

Fig. 17.—Dog No. 8, Exp. 4; roentgenograms of fore and hind legs. Animal 10 months and 1 day old. Period of observation after operation, nine months and twelve days.

Fig. 18.—Control dog No. 8, Exp. 4; 10 months 1 day old. Period of observation, nine months and twelve days. Note that the bones of dog No. 8 are more slender than those of No. 8, control, but show no abnormalities.

Fig. 19.—Control dog No. 39, Exp. 8. Roentgenogram taken when 14 months and 15 days old. Period of observation, thirteen months and twenty-six days.

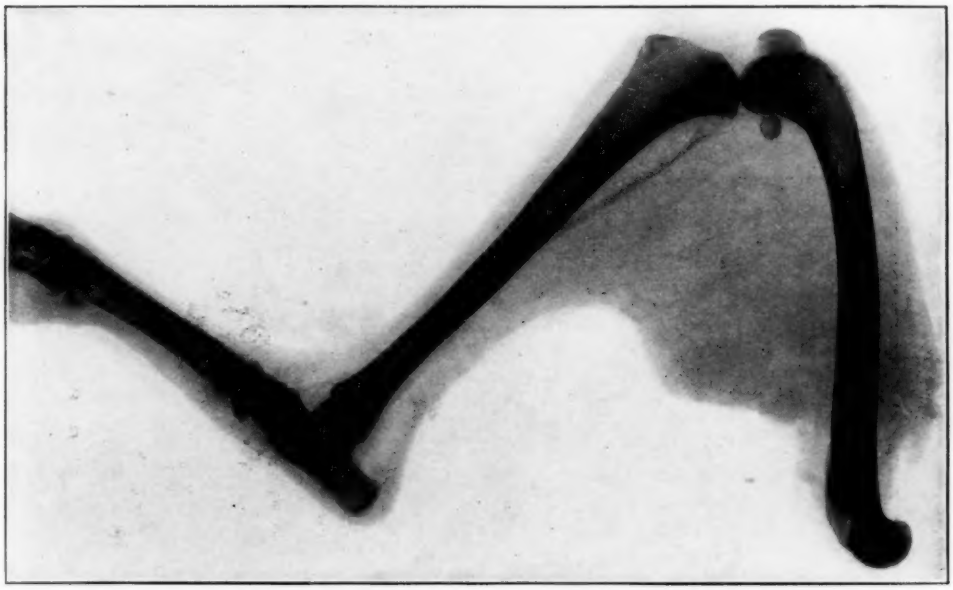

Fig. 20.—Thymectomized dog No. 39, Exp. 8. Roentgenogram taken when 14 months and 15 days old. Period since operation, thirteen months and nineteen days.

Fig. 21.—Thymectomized dog No. 40, Exp. 8. Roentgenogram taken when 14 months and 15 days old. Period since operation, thirteen months and twenty-five days. Note that the bones of both thymectomized animals are normal.

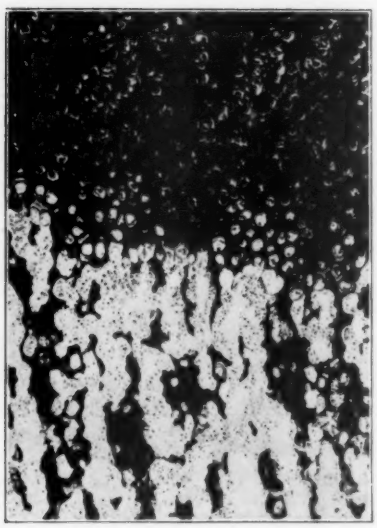

Fig. 22.—Dog 75 (rib)

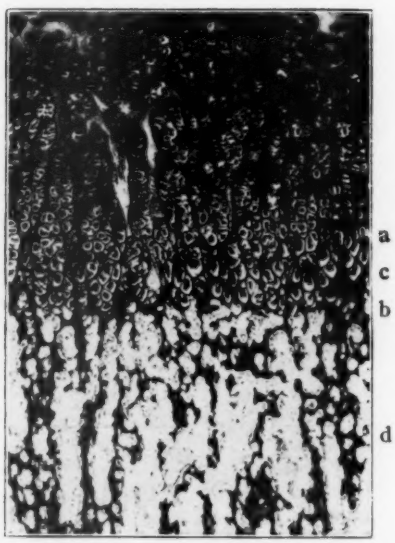

Fig. 23.—Dog 70 (rib)

Fig. 22.—Photomicrograph of rib of dog No. 75, Exp. 14, thymectomized when 13 days old. Period of observation, one month and twenty-seven days. Low power magnification. (a) Proliferating cartilage normal; (b) calcium deposition in cartilage abundant; (c) trabeculae normal. The section is cut somewhat obliquely to the long axis of the rib; for that reason the line of junction of the cartilage and the shaft does not appear to be perfectly straight.

Fig. 23.—Photomicrograph of rib of dog No. 70, Exp. 13; showing costo-chondral junction. Low power magnification. Thymectomy performed on the fifteenth day. Period of observation, five months and seventeen days. (a) Cartilage columns normal; (b) line of junction of cartilage and shaft clearly defined; (c) calcium deposition in cartilage normal; (d) trabeculae normal. No sign of rickets. (Decalcified by liquor formaldehydi-formic acid method. Stained with hematoxylin and eosin.)

Fig. 24.—Dog 8 (rib)

Fig. 25.—Dog 8 (femur)

Fig. 24.—Photomicrograph of rib of dog No. 8, Exp. 4, showing junction of cartilage and shaft. Low power magnification. Thymectomy performed on the nineteenth day. Period of observation, nine months and twelve days. (a) Cartilage in parallel columns; (b) calcium deposition in cartilage normal; (c) trabeculae of shaft normal. No sign of rickets. (Decalcified by liquor formaldehydi-formic acid method. Stained with hematoxylin and eosin.)

Fig. 25.—Photomicrograph of junction of epiphysis and diaphysis of lower end of femur of thymectomized dog No. 8, Exp. 4. Low power magnification. Thymectomy performed on the nineteenth day. Period of observation, nine months and twelve days. (a) Epiphysis; (b) band of proliferative cartilage; (c) trabeculae of shaft. No sign of rickets. Decalcified by liquor formaldehydi-formic acid method. Stained with hematoxylin and eosin.

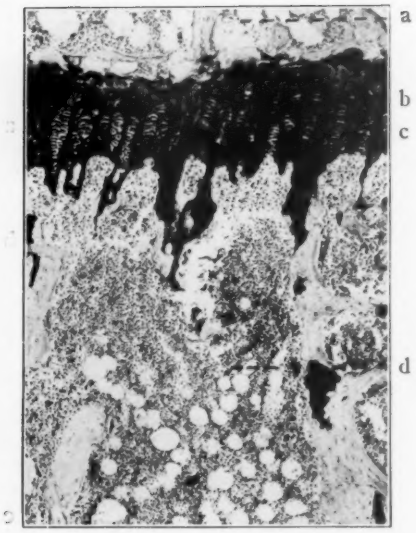

Fig. 26.—Dog 8 (femur)

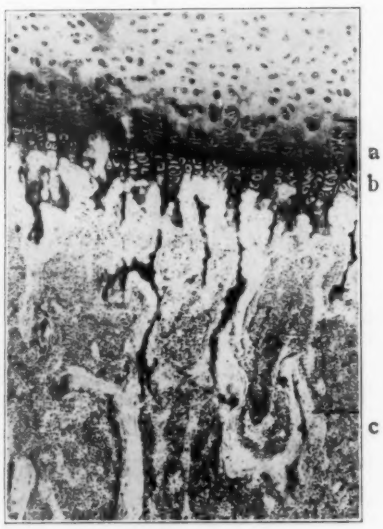

Fig. 27.—Dog 62 (rib)

Fig. 26.—Photomicrograph of junction of epiphysis and diaphysis of the lower end of femur of thymectomized dog No. 8, Exp. 4. Low power magnification but higher than that in Fig. 25. (a) Epiphysis; (b) band of proliferative cartilage; (c) calcium deposition in cartilage; (d) diaphysis. No sign of rickets. Decalcified by liquor formaldehydi-formic acid method. Stained with hematoxylin and eosin.

Fig. 27.—Photomicrograph of rib of thymectomized dog No. 62, Exp. 11. Low power magnification. Thymectomy performed on the twentieth day. Period of observation, one year. (a) Proliferative cartilage; (b) calcium deposition in cartilage; (c) trabeculae of shaft. Line of junction of cartilage and shaft is clearly defined. No sign of rickets. Decalcified by liquor formaldehydi-formic acid method. Stained with hematoxylin and eosin.

almost four months after operation stands, however, in flat contradiction to Klose's statement that from a minute fragment the thymus regenerates to the size of the original gland, and indicates that, even if the thymus has an unusual regenerative capacity, Klose's statement of it must be the grossest kind of exaggeration.[157] If virtually the whole thymus reappeared again after the main mass had been removed, that startling fact could never have escaped the poorest investigator. The older literature, and, in fact, the entire experimental literature, would have constituted one grand succession of proofs of the extraordinary regenerative power of the thymus gland. It would not have been necessary to search for thymus rests in the thyroids and in the necks of thymectomized animals with microtome and microscope; they would have revealed their presence by the formation of visible and palpable tumors in the living animals. All question whether a thymectomy was complete or not would vanish, if great masses of thymus tissue failed to develop in the thorax or neck. We believe, however, that the history of thymus extirpation has shown that the power of the thymus to regenerate is distinctly limited. We doubt whether the thymus rest left at operation doubles its volume as the result of any regenerative change, and should not be surprised if it were found that it did not regenerate at all. The whole subject of regeneration of the thymus rest must be reinvestigated. The question can never be settled in the aimless way (Fulci's work excepted), in which it has been treated in the past.

THE INFLUENCE OF THYMECTOMY ON LIFE

All of our thymectomized and control dogs, with the exception of two, dogs Nos. 5 and 62, control, are available for analysis in regard to the influence of the removal of the thymus gland on life, although a number, which died from intercurrent disease comparatively early in the experiments, have a very limited value from that standpoint. Dog No. 5 will not be considered because in him a rest of normal thymus as large as a pea was found at necropsy, and dog No. 62, control, because death occurred early from a traumatic cause (drowning).[158] We have not dismissed from consideration dog No. 30, Exp. No. 6, in which a minute rest of thymus tissue was purposely left at operation,

157. It is difficult to understand how Klose can maintain, on the one hand, that the thymus regenerates completely from a minute rest and, on the other hand, that the negative results of other investigators were due to the incompleteness of their thymectomies when they found no thymus tissues even on microscopic examination at necropsy. Even if the thymus rest involuted after it had attained practically the size of the entire gland, its remnants would be unmistakable.

158. The dog had congenital cataract and was blind. He fell into a well and was drowned.

and tissue thought to be atrophied thymus found at necropsy almost eleven months afterward for the reason that the cause of death of that dog was not well understood, as will be explained presently. We have not excluded dogs Nos. 37 and 38, Exp. No. 7, in which very minute thymus rests were left at operation, because no vestige of them could be found at necropsy after the most careful examination of the serial sections (see protocol of experiment).

With dogs Nos. 5 and 62, control, eliminated, there remain forty-one dogs, of which twenty-three were thymectomized and eighteen were control animals. Of the twenty-three thymectomized dogs, eleven, or 48 per cent., died from natural causes, as compared with six of the eighteen control animals, or 33 per cent. These figures do not exactly represent the truth, however, for one of the thymectomized and one of the control dogs were in a moribund condition when sacrificed. If, then, we consider those two animals as having died of disease, the figures would stand twelve out of twenty-three thymectomized dogs, or 52 per cent., dying from natural causes as opposed to seven out of eighteen, or 39 per cent., of the control animals. Thus it is seen that the mortality among the thymectomized animals was 13 per cent. higher than among the control animals. The percentage difference just given, however, cannot be assumed to indicate that abolition of thymus function exerts an influence inimical to life for the reason that another factor, equally undetermined, must be taken into account, namely, the effect of the operation itself. The rôle which the operation may play in the causation of underdevelopment, sickness and death will be discussed more fully in the chapter entitled "The Influence of the Removal of the Thymus on Nutrition and Growth." It is sufficient for the present to remark that the preponderance of 13 per cent. in the mortality of the thymectomized animals is certainly no greater than might have been expected as the result of the factor introduced by the operation itself.

If we turn now to the group of eleven thymectomized dogs which died natural deaths, we are able to explain the deaths of all from causes unrelated to loss of thymus activity with the possible exception of dog No. 30.

Dogs Nos. 10, 60 and 61 died as the result of chronic infection with round worms, seven months, two and one half months and two and one half months, respectively, after operation. (The control mates of dogs Nos. 60 and 61 died coincidently as the result of round worm infection. The control mate of dog No. 10 died from distemper.)

Dog No. 38 acquired an infection of the thoracic wall at the time of operation which persisted with sinus formation. Probably as the result of the infection he never completely recovered from the oper-

ation, and remained weaker than the other two dogs of the litter, thymectomized dog No. 37 and the control dog, No. 37-38, control. He died a little more than one month after operation during an unexpected period of intense winter cold. The reason for thinking that the cold brought about the dog's death was that the other two stronger dogs of the litter were severely affected and barely survived.

Dog No. 67 died as the result of pneumonia two months and nineteen days after operation. [His control mate, dog No. 67, control, was suffering from a respiratory infection acquired coincidently when sacrificed.[159]]

Dog No. 43 died as the result of pyemia complicating mange one month and twenty-seven days after operation. (The control of dog No. 43 died simultaneously from the same cause.)

Dogs Nos. 71, 72, 74 and 75 died one month and twenty-seven days, one month and fifteen days and one month and twenty-seven days, respectively, after operation as the result of conditions entirely unrelated to loss of thymus, and the illness of dog No. 70, the animal which was moribund when killed with ether five months and seventeen days after operation, was also obviously not dependent on loss of thymus activity.[160]

The nature of the illness of dog No. 30, experiment No. 6, was obscure. She gradually emaciated and died eleven months after oper-

159. Both dogs, Nos. 67 and 67, control, were perfectly healthy animals until accidentally left out of doors one warm night in March. During the night there was a sudden fall in the mercury accompanied by a snow storm. Both dogs developed a respiratory infection. The sickness of dog No. 67 lasted three days.

160. As has been stated already, we placed the last four litters of our experiments in the homes of private families with the expectation that the dogs would there receive individual care and escape the severe infection with round worms, mange and distemper which had complicated our experiments at the farm and which are inevitable when large groups of young dogs are confined together for long periods of time. Accordingly, we put litter No. 13, composed of dogs Nos. 70 and 70 C and litter No. 14, composed of dogs Nos. 71, 72, 73, 74, 75 and the two control animals in the home of laboratory janitor D. As events ultimately proved we made a mistake in placing the animals in D.'s charge. In the first place the yard behind D.'s house was so small that the animals were thrown into even closer contact with each other than at the farm. Dog No. 70, control, was sick with a severe infection with round worms which did not yield to treatment and ultimately caused the animal's death. The young animals of litter No. 14, only 2 weeks old, and not yet recovered from the operation, were thus brought into the closest contact with dog No. 70, control, and acquired a severe infection with round worms. In the second place, it was discovered afterward that D. gave the animals the poorest care. He fed them canned fish because it was the cheapest available food, instead of the prescribed diet and administered repeatedly, without discrimination, a patent worm cure. To some of the animals he gave daily doses. Of the six thymectomized and three control dogs under D.'s care, every dog sooner or later became sick with the exception of one control animal. Four thymectomized and one control dog died.

ation, and at necropsy showed no pathologic condition except blood and mucus in the intestine. She had been treated shortly before death for infection with round worm. We thought that death in her case was due to a prolonged diarrhea associated with ascaris infection, but cannot be certain for lack of sufficiently careful observations.

Thus it seems possible to explain the sickness and deaths of all the thymectomized dogs on other grounds than deprivation of thymus function, with the possible exception of one animal, dog No. 30. In the case of dog No. 30 we cannot be certain that death did not occur as the result of the removal of the thymus gland. If all the thymectomized dogs of our experiments, or almost all of them, had behaved like this animal, and the control dogs had remained normal, we should not have hesitated to assign the cause of death to the deprivation of thymus secretion. The skeleton of dog No. 30 was, however, normal on microscopic examination and the suprarenals and thyroids showed no definite changes. Indeed on histologic examination of the tissues of dog No. 30 no changes were found in any of the organs which were different from those found in the control dogs or in the other thymectomized animals.

Turning now to the other group of ten thymectomized dogs which remained well throughout the course of the experiments, all were observed for more than seven months after thymectomy without showing any evidence that deprivation of thymus function influenced life; eight were observed for more than nine months, six for one year or more, three for thirteen and one half months or more, and one for fifteen months. Moreover, the tissue from the thorax and neck of this group of dogs which in any way resembled thymus, or was thought possibly to contain thymus, was examined with the utmost care, in serial sections for rests, except in two instances, and no thymus tissue was found.

If the thymus gland is necessary to life, then we should have expected from the negative results of our systematically conducted

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Two thymectomized and one control dog were sick when killed with ether. (See protocols of experiments on litters Nos. 13 and 14.)

Dr. W. Sisson, who previously had experimental and control puppies at D.'s house under D.'s care, had an experience exactly similar to ours. He lost every one of his control as well as experimental animals as a result of diarrhea and ascaris infection.

It is important to note that of the eleven thymectomized dogs dying as a result of disease all but two, or 82 per cent., died within the first two months. The shortest interval after removal of the thymus at which death can be ascribed to cessation of thymus function is, according to Klose, three months. None of the thymectomized dogs of these two litters showed any pathologic changes in the skeleton or organs which distinguished them from the control animals. (See protocols of experiments.)

histologic examinations of the tissues taken from the neck and thorax that not 13 per cent. more thymectomized dogs than control animals would have died, but that all the thymectomized dogs, or all with a very few exceptions, would have died. Furthermore, we should have expected that death would have been preceded in the case of each animal by the development of a sickness, or at least of certain pathologic manifestations, at about the same interval after removal of the thymus gland, and that the sickness or the pathologic manifestations would have been true to some common type, and as characteristic of the absence of thymus function as myxedema is of the absence of thyroid function, or as eunuchism is of the absence of testicular function, or as the acute manifestations which follow removal of the parathyroids or suprarenals are characteristic of the loss of function of those organs of internal secretion. Moreover, it would have been reasonable to suppose that there would have been characteristic pathologic changes either visible to the naked eye or present on microscopic examination, which would have distinguished the thymectomized from the control animals. But no pathologic changes could be found in the bodies of those thymectomized animals which died natural deaths or of those sacrificed other than those of the diseases from which they suffered. Accordingly, we believe that it is necessary to disregard the case of the single animal, dog No. 30, whose sickness and death cannot be explained satisfactorily, and to draw the conclusion that the function of the thymus gland is absolutely not essential to life.

THE INFLUENCE OF REMOVAL OF THE THYMUS ON NUTRITION AND GROWTH

For the study of the influence of thymectomy on nutrition and growth the dogs of our experiments may be divided into three groups. Group 1 is composed of eight pairs of thymectomized and control dogs from different litters (Nos. 1 and 1 C, litter No. 1; Nos. 4 and 4 C, litter No. 2; Nos. 7 and 7 C, litter No. 3;[162] Nos. 8 and 8 C, litter No. 4; Nos. 13 and 10 C, 12 and 12 C, litter No. 5;[163] Nos. 39 and 39 C, 40 and 40 C, litter No. 8) which either remained entirely free from disease throughout the course of the experiments or else were affected later, months after the thymus had been removed. The period of observation after operation varied between seven months and six days, and fifteen months and thirteen days. The dogs of Group 1 form,

162. Dog No. 5 of this litter was not included because he remained normal throughout the period of observation of four months, and at necropsy was found to have a rest of normal thymus tissue.

163. Dog No. 10 of this litter was not included because of chronic disease (infection with round worms). Dog No. 13, control, was not included for the same reason; he died with ascaris infection when 4 months old.

therefore, excellent subjects for the study of the problem under consideration. Group 2 is composed of five pairs of dogs from five litters (Nos. 30 and 30 C, litter No. 6; Nos. 37 and 37 C, litter No. 7; Nos. 62 and 62 C, litter No. 11; Nos. 67 and 67 C, litter No. 12; Nos. 70 and 70 C, litter No. 13). The dogs of Group 2 are of much less value than those of Group 1 because of the comparatively short period of observation (litters Nos. 12 and 13), incomplete weight records (litters Nos. 6 and 7), and the comparatively early death of the control (litter No. 11). Group 3 is composed of all the dogs not included in Groups 1 and 2. The animals of Group 3 are valueless for the determinations of the influence of thymectomy on growth and development because of the presence among them of disease, which obviously had no relation to deprivation of thymus function, dating from the beginning of the experiments. They will not be considered in this connection.

Turning now to the eight pairs of thymectomized and control dogs comprising Group 1, we find that the thymectomized animal maintained his weight, as compared with the control animal in the case of six, or 75 per cent. of the pairs (dogs Nos. 1 and 1 C, 4 and 4 C, 7 and 7 C, 13 and 10 C, 12 and 12 C, 40 and 39 C) [See Figs. 8 and 9], but fell behind the control in weight in the case of the remaining two pairs, or in 25 per cent. (Nos. 8 and 8 C, 39 and 40 C). In none of the pairs of this group did the thymectomized dog outstrip the control in weight.

In one of the two pairs in which the thymectomized animal fell behind the control in weight the difference became exceedingly marked (Fig. 10). Thymectomized dog No. 8 was only 3 per cent. lighter in weight than No. 8, control, on the thirteenth day of life when the thymus was removed, but a little less than a month after operation he was 22 per cent. lighter, five months after operation he was 32 per cent. lighter, and when killed, nine and one half months after operation, he weighed a little less than half as much as No. 8, control. Whereas, dog No. 8, control, gained steadily from the beginning to the time of his death, thymectomized dog No. 8 reached his maximal weight five months after operation, and then remained essentially stationary in weight. Necropsy findings indicated, however, that growth in the case of dog No. 8 was still in progress, but had ceased in dog No. 8, control, as will be explained later. In the case of the other pair (thymectomized dog No. 39 and No. 40, control) in which the thymectomized animal did not gain weight so rapidly as the control, the difference was not nearly so great as between dogs Nos. 8 and 8, control, just mentioned. At the time of operation, on the nineteenth day of life, dog No. 39 weighed 13 per cent. less than No. 40, control, one month after operation he weighed 24 per cent. less, and six and one

half months after operation he weighed 28 per cent. less. There was, moreover, a great difference in the weights of the two control dogs of this litter (23 per cent. six months after the operation). If, instead of comparing thymectomized dog No. 39 with control dog No. 40, control, we had compared thymectomized dog No. 39 with the other control dog No. 39, control, we would have found the difference in weight to be comparatively slight[164] (7 per cent. nine months after operation). In the presence of so wide a range of normal variation in weight among the animals of this litter, as is indicated by the great difference in the weights of the two control dogs, and the slight difference which existed between the weights of dogs Nos. 39 and 39, control, the marked difference in weight between dog No. 39 and dog No. 40, control, loses practically all of its significance.

Turning to the consideration of the five pairs of thymectomized and control dogs of the less important Group 2, we find that the thymectomized dog No. 30, which was slightly heavier than No. 30, control, at the time of operation, weighed less than the latter three months after operation, and at the end of four months had fallen behind No. 30, control, to the extent of 12 per cent. When dog No. 30 died, one year after operation, she was greatly emaciated as well as much smaller than her control mate, and undoubtedly weighed much less than No. 30, control. Exactly when cessation of growth or actual loss of weight occurred in the case of dog No. 30 we do not know, because of the absence of weight records after the fourth month.[165] Dog No. 67 (Exp. 12) weighing 12 per cent. less than No. 67, control, at the time of operation, gained less rapidly than the latter from the first, and after death, which occurred two months and nineteen days after

164. On the day of operation, thymectomized dog No. 39 weighed 20 per cent. more than No. 39, control, but fell behind the latter during the first two weeks following the operation, probably as the result of the operation. One month after operation dog No. 39 weighed 12 per cent. less than No. 39, control, but six and one-half months after operation he weighed only 7 per cent. less.

For the first six and one-half months after operation thymectomized dogs Nos. 39 and 40 gained steadily in weight, in contrast to dog No. 8 of litter No. 4. At the end of six and one-half months dog No. 40, control, was the heaviest of the four animals of the litter, thymectomized dog No. 40 came next, control dog No. 39 next, and last of all thymectomized dog No. 39. Thus, thymectomized dog No. 40 was heavier than one control and lighter than the other control. The weights of the dogs of this litter were not taken during the last seven months of the experiment (except after the animals had been killed) because of distemper which broke out among them, killing dog No. 40, control, and causing the other three dogs to remain sick for so long a time that at the end of thirteen and one-half months, when they were sacrificed, only one of them (dog No. 39, control) weighed as much as seven months previously.

165. Dog No. 30 was neglected because thymectomy in her case was incomplete; the experiment in which she figured was regarded as unimportant.

operation, weighed 36 per cent. less than No. 67, control.[166] Dog No. 37 (Exp. 7), on the other hand, weighing about the same as his control dog at the time of operation, exceeded the control dog in weight by 18 per cent. when killed a year later. Dog No. 70, also, which was considerably heavier than the control dog at the time of operation, gained more rapidly than the latter from the very beginning, and at the time of the death of dog No. 70, control, which occurred three months after operation, weighed more than twice as much. The weight curves of the last remaining pair of this group, dogs Nos. 62 and 62, control, (Exp. No. 11), remained exactly parallel for two and one half months after operation, when the control animal met an accidental death. The thymectomized dog continued to gain at a normal rate and weighed 6.8 kg. when killed one year after the thymus had been removed. Thus, of the dogs in Group 2 the thymectomized animal grew less rapidly than the control in point of weight in two instances, more rapidly in two instances, and in one instance at the same rate as the control dog during the duration of the life of the latter.

Thus it is apparent that the results of our experiments which bear on the question of the influence of deprivation of thymus function on growth as measured in terms of weight are not uniform. If the animals of Groups 1 and 2 are considered together, in a little more than half the experiments (7) no essential differences in weight could be demonstrated, but in the remainder (6) differences were present, the less rapid weight increase in two experiments being exhibited by the control, and in four by the thymectomized dogs.

Since we made no measurements of the length or height we were obliged to rely entirely on impressions as to differences in size and also in the nutritional state of the two sets of animals. All that it is possible to say in regard to these matters is that as compared with their control animals the thymectomized dogs did not appear to be tall or short, thick set or slender, or to have more abundant or less abundant or more firm or less firm musculatures. The thymusless dogs weighing less or more than their control mates appeared to be simply smaller or larger, normally proportioned animals. One exception must be made to this statement, however. Dog No. 8 (Exp. 4), which showed such marked underweight as compared with his control mate, was a perfectly formed, vigorous, extremely active animal, and when seen alone would have been considered a normal dog, but seen with his control dog appeared extraordinarily slenderly built, with narrow chest and haunches, delicate limbs and scant musculature (Fig. 11).

166. Allowance must be made for the fact that dog No. 67 must have lost considerable weight in the course of the acute illness, pneumonia, which caused his death. The weight from which the 36 per cent. difference was calculated was obtained postmortem.

It is clear that our experiments cannot be interpreted like those of MacLennan[83] as indicating that removal of the thymus has a stimulating influence on growth. Not only did a very great minority of the thymusless animals (two, or 14 per cent.) reach weights in excess of those of their control animals, but the more striking of the two experiments (Exp. 13), in which the thymectomized dog grew to be more than twice as heavy as the control animal, must be excluded from consideration. In that experiment dog No. 70, control, was sick from round worm infection and died as the result. But even if the growth of dog No. 70, control, had not been complicated by disease, a failure of correspondence in rate of growth between him and the thymectomized dog would not have been remarkable; for the two animals were so different in coloring, shape of ears, length of hair, etc., as to indicate a very different inheritance, and to make marked disproportion in size in no wise remarkable. On the other hand, we do not believe that our experiments can be interpreted to mean that deprivation of thymus function exercises a retarding influence on growth and development. It is probable that the smaller weight of dog No. 30 was to be attributed to disease unrelated to loss of thymus function, and the smaller weights of the other three thymectomized dogs to individual variation or to the influence of the operation itself, or to both causes. The evidence in favor of the view that individual variation may have been the determining factor in respect to the differences in the weights of dogs Nos. 8 and 8, control, and Nos. 39 and 40, control, is considerable.[167]

The fact that our dogs were not of thoroughbred stock, standardized in point of size, impairs the value of our experiments in their bearing on the question of the influence of thymectomy on growth. It is a well known fact that far greater variations in growth occur in the offspring of mongrel than in the offspring of thoroughbred stock

167. Dog No. 8, the most marked example of undergrowth afforded by our experiments, differed much, even at birth, from No. 8, control. His hair, for example, was long and shaggy, whereas that of the control dog was short and smooth. His nose was square, but that of the control animal pointed. No one would have suspected that the two dogs were brothers (Figs. 11, 12 and 13). In experiment No. 8, in which the undersized dog No. 39 figured, two of the dogs, Nos. 40 and 40, control, had short hair, and two, Nos. 39 and 39, control, long hair (Figs. 14 and 15). The two short haired dogs grew faster than the two long haired dogs. The retardation in growth shown by the long haired thymectomized dog, No. 39, was comparatively slight when measured solely in terms of the growth of the long haired control, dog No. 39, control. As already pointed out, six and one half months after operation when thymectomized dog No. 40 died (distemper pneumonia) dog No. 40, control, was the heaviest of the four animals in the litter; thymectomized dog No. 40 ranked next, then control dog No. 39, and last of all thymectomized dog No. 39. Thus, the thymectomized dog was heavier than one control and lighter than the other.

(Davenport,[168] Castle,[169] MacDowell[170]). But even in thoroughbred stock, bred particularly with a view to uniformity in size of offspring, for example, fox terriers and beagles, it is not uncommon to find one animal of a litter much smaller than any of the others, colloquially known as the "runt." The tremendous importance of the use of standardized stock in any experiments in which the growth and nutritional state of the animals are the final criteria, has been greatly emphasized in recent experimental work, and cannot be emphasized sufficiently. Only in case, therefore, all or almost all the thymectomized dogs of our experiments had shown the same well marked variation in growth as compared to the control animals, in other words, unless the evidence had been overwhelming, would we be justified in drawing the conclusion that deprivation of thymus function influenced growth.

There are other factors, however, than chance variation which must be considered. The operation of thymectomy as performed in the dog, involving artificial respiration, double pneumothorax, exposure and manipulation of the heart and lungs, the great vessels and nerves of thorax and neck, is as severe a test of the vitality of the animal as any operation that could be mentioned. Yet the opinion held among investigators of this problem seems to be that the operation ceases to have any significance insofar as the future development of the animal is concerned, once it has been survived. There can be no doubt, however, that the operation, directly or indirectly, may be the cause of prolonged retardation in growth and development. All writers appear to agree that the operation of thymectomy may be responsible for a very temporary retardation in growth and development. At the end of four days, in our experience, it was usually possible to say that the animal had recovered from the operation in the sense that it had then become evident that he would survive. Not for two weeks or more did the thymectomized puppies become indistinguishable from the control puppies in general behavior and appearance, and in some of our experiments the operated animals never did recover from the initial setback of the operation (dog No. 38, Exp. 7; dogs Nos. 72, 73, 74 and 75, Exp. 14).

The operation of thymectomy may be the cause of continued retardation of growth and development in different ways. In some animals a focus of infection persists which is sufficient to delay normal develop-

168. Davenport's work indicates that the inheritance of stature follows the Mendelian law. Davenport, C. B.: Inheritance of Stature, Genetics, Princeton, N. J. **2**:313, 1917.

169. Castle, W. E.: Studies of Inheritance in Rabbits, Washington, D. C., 1909, Carnegie Institute.

170. MacDowell, E. C.: Size Inheritance in Rabbits, with a Prefatory Note and Appendix by W. E. Castle, Washington, D. C., 1914, Carnegie Institute.

ment, though not sufficient to make the animal appear sick. The focus of infection may be recognized during life (dogs Nos. 10 and 38), or not until necropsy (dog No. 74), or may, without doubt, disappear after a time, so that at necropsy nothing is found to awaken suspicion that any cause for retardation of growth other than deprivation of thymus function could have been present. In other thymectomized animals disease develops in the period in which the resistance remains lowered as the result of the operation, and becomes so established that the initial retardation is perpetuated, and the operated animal remains weak and backward in growth in comparison with the control animal. Among the morbid conditions which frequently do obtain a foothold during the postoperative period may be mentioned infection with round worms, mange and diarrhea. The initial setback caused by the operation may be prolonged indefinitely as the result of unfavorable environmental conditions. It is a well known fact that dogs bear confinement poorly. If, following operation, the weakened dog is thrust into a cage or confined in a cellar (Matti's animals), or in a drygoods box in a dark stable (the reported method of treatment of a well known investigator), or if he is given improper food or is exposed to cold, his continued underdevelopment or even sickness and death—the control animal remaining healthy the while—is not surprising. The explanation of the retardation of growth of the five thymectomized puppies of experiment 14 and of their sickness leading to death within one and three fourths months after operation, while the two control animals survived,[171] lay in the fact that they were the weakest animals of the litter, fresh from the operation, and only thirteen days old when thrust into most unfavorable environmental conditions. The first two series of experiments of Nordmann,[121, 122] in which the thymectomized dogs developed pyodermia, alterations in the hair, etc., and finally died unexplained deaths with dilated hearts, and the control dogs remained normal, must be interpreted in a similar manner; for in his third series of thymectomy experiments[124] environmental conditions were so improved that the infection, etc., did not occur, and no changes of any sort developed in the thymectomized dogs. The temporary retardation of growth which Basch reported may well have been due to the operation and confinement. In fact, we should not be surprised if all examples of underdevelopment, illness and death of thymectomized animals which have been attributed to loss of thymus function had their actual foundation, if not in chance variation, in the effects of the operation itself plus one of the factors mentioned. Human medicine presents too many analogies of the prolonged retarding effects of severe

171. One of the two control animals was sick, and probably would have died if he had not been killed.

operation (for example, the operation for harelip and cleft palate) on the growth and development of infants, particularly very young infants and those placed in unhygienic surroundings, for us to doubt that the severest kind of operation may have similar consequences in the puppy.

Our experiments, as a whole, fail, therefore, to indicate that destruction of the thymus function influences nutrition and growth. A very great minority of our experiments, however, suggest the possibility that it has a depressing or retarding effect on growth and development.

THE INFLUENCE OF THYMECTOMY ON THE GROWTH OF THE HAIR, THE DEVELOPMENT OF THE TEETH, THE FORM, GAIT, INTELLIGENCE AND APPETITE

The thymectomized dogs of our experiments failed to show any abnormality in the growth of the hair. Though the coats of several of them were thicker and more shaggy than the coats of their control dogs, exactly the reverse condition obtained in other pairs of animals. We observed that the hair of several dogs (dogs Nos. 10, 30 and 38), which were sick and greatly malnourished, lost its luster, became exceedingly dry and tended to stand up in places, giving the animal an unkempt appearance. Klose and others described alterations of the hair of a similar nature in the thymectomized dogs of their experiments, and have interpreted them as a trophic disturbance, specifically caused by the abolition of thymus activity, analogous to the changes in the hair which occur in myxedema. It was impossible, however, to regard the abnormal condition of the hair in the few animals of our experiment showing it as related in any way to loss of thymus function, if for no other reason, because it was present in two of the control animals. It was clearly a part of the marantic state of the animals. Even if some of the many changes which Klose reported in his thymectomized dogs were actually due to deprivation of thymus function, it appears to us very doubtful whether any justification existed for arbitrarily includng among them the alterations in the hair occurring during the "stage of cachexia."

Observations on the teeth of our thymectomized and control dogs were not conducted in a routine manner. Inspections of the mouth from time to time failed to reveal, however, the slightest evidence of any disturbance, such as Klose has recently described, in the time of eruption or in the form of the teeth of the thymectomized dogs.

The dogs deprived of thymus were normally formed. Not one of them exhibited any bowing of the forelegs, enlargements about the joints, valgus conditions of the hind legs, atrophy of the hind quarters, beading of the ribs, etc., such as described by Basch, Klose and Matti

in their thymectomized animals. Several thymusless dogs did show at necropsy deformity of the thorax in the nature of pigeon breast, which, however, was so plainly the result of faulty union of the parts of the sternum divided at operation as to require no further comment.

The gait and the posture assumed by the thymusless animals in standing and sitting did not differ from the gait and posture of the control animals except in a few instances. Several of the thymectomized dogs walked awkwardly with forelegs spread apart for a short period after operation, having a gait which, perhaps, might have been termed "straddling." When, however, the nature of the operation to which the animals had been subjected was considered, involving incision through the sternum, the widest possible separation of the divided parts of the thorax, injury to the pectoral muscles and those of the neck, and finally an apposition and union of the divided parts of the sternum, which was perhaps faulty or had become loose as the result of movements of the animal, it seemed remarkable that disturbances in gait, involving particularly the forelegs, had not been a more common sequel to the operation. At the end of from two to four weeks the changes described in the gait had disappeared entirely. None of the animals walked or stood in the flat-footed position described by Basch (Fig. 2), Klose and Matti, or in the semi-crouching position described by Matti, and regarded by these workers as typical. A dog came under our notice, however, which did show the identical postural abnormality of the feet mentioned by the writers just named, as a characteristic development in the thymectomized dog and in addition marked thickenings of the bones about the joints and marked bowing of the forelegs (Fig. 3). This dog showed every deformity exhibited by the living thymectomized animals of Basch, Matti or Klose, and in as severe a form (judging from the illustrative photographs) as the worst affected of them. Indeed, the photograph of this dog might have been substituted by Matti or Klose for any of their illustrations purporting to show the pathologic changes developng in the living animal as a consequence of removal of the thymus. Yet this animal was never subjected to any operation. His thymus gland was never touched. He was merely confined in a cage 4 by 4 feet for three months, beginning with the sixth week of life, and fed a mixture of meat and cornmeal. At the necropsy his thymus gland weighed 2.8 gm. (body weight, 9.4 kg.). The long bones of the legs were so fragile that it was difficult to section them without producing fracture of the shaft. The teeth were loose. (At the time of going to press it is impossible to state whether or not the animal had rickets, because the microscopic sections are not yet ready.) Another dog from a different litter (Fig. 16) fed on a diet composed of milk, bread and meat, and confined in a cage for two

months after weaning was complete, developed well marked prominences at the knee joints and slight bowing outward of the forelegs. The thymus of this dog weighed 2.5 gm. The skeleton at necropsy was free from rickets, but showed well marked osteoporosis. This dog had never been subjected to any operation.

We have called attention to the occurrence of deformities in the legs of these two dogs in order to emphasize the fact that deformities exactly like those described by Basch, Matti or Klose as occurring in thymectomized dogs may develop as the result of confinement alone or of confinement and dietetic influences. It cannot be repeated too often that gross alterations in the limbs, such as enlargements about the joints, flat foot, curvature of the long bones, accompanied by diminution in the resistance of the bone to bending or to cutting, and with necropsy findings of osteoporosis, or rickets, though occurring in thymectomized dogs, have no specific significance.

We could not detect any disturbance in the intelligence of the thymectomized dogs as compared with the control dogs, and are certain that none which could be attributed to deprivation of thymus function existed. Toward the close of a sickness of some duration one of the thymectomized dogs (dog No. 10) became stupid in appearance and sluggish in movement. With the exercise of a little imagination it might have been said of that animal, as Klose and Vogt wrote in regard to the thymectomized dogs of their experiments in the last stages of their illnesses, that he "failed to show signs of recognition of his keeper" or "of knowing when the feeding hour was due," that he "soiled his kennel," and that "he did not come when called." It might have been possible to have described the "gaze" of this animal as at times "dreamy," and the "expression" as "senile." We could not interpret the behavior or appearance of this dog, however, as indicating more than an extreme depression of cerebral function, the result of disease. If he had recovered from the infection with round worms which caused his death, we should have expected a return of the normal signs of intelligence. We cannot but suspect that the evidences of disordered intelligence which Klose and Vogt brought forward as proofs of a condition of "thymic idiocy" were nothing more than the ordinary behavior of the dog in the terminal stages of severe disease.

It is certain that none of the dogs of our experiments showed any perversion of appetite which could be ascribed to the loss of thymus function. In the stomachs of one thymectomized and two control dogs (all three were sick animals) were found at necropsy foreign bodies, such as stones and sand. The ingestion of foreign substances is a common accompaniment of disease in the dog, and certainly does not point to a disturbance of thymus function, as Klose and Vogt and

others pretend. The thymusless dogs of our experiments did not have more voracious appetites than their control mates. Unlike Klose and Vogt's thymectomized animals, also, none of our dogs mutilated or injured themselves.

THE EFFECT OF REMOVAL OF THE THYMUS ON THE SKELETON OF THE DOG

For the study of changes in normal growth and development of the skeleton after loss of the thymus obviously only those of our experimental dogs were available which remained free from intercurrent disease while growth was in progress. It so happens, however, that all the animals of our experiments, whether diseased or not, are available for the inquiry into the occurrence of pathologic developments in the skeleton of thymoprivic origin, as will be explained presently.

The question of the relation of thymectomy to growth of the skeleton in the dogs of our experiments in reality has already been considered in the discussion of the influence of removal of the thymus on the growth and development of the animal as a whole. The four dogs of Groups 1 and 2[172] (dogs Nos. 8, 39, 30 and 67), which were smaller than their control dogs, had smaller skeletons in the same proportion as they themselves were undersized, and the leg bones and skulls of one of them (dog No. 8) placed side by side with the corresponding bones of his control mate might have been used to furnish a striking demonstration of the depressing influence of removal of the thymus gland on the growth of the skeleton. As was pointed out in the discussion just referred to, however, it was plainly inadmissible to assume that the undersize of the four animals in question was caused by deprivation of thymus function to the exclusion of certain other factors. Certainly no justification existed for selecting the four experiments in which retarded growth of the skeleton occurred, as representing the truth merely because the results in them were of a positive character, and for rejecting the nine experiments in which retarded growth did not occur merely because the results were negative, when every bit of evidence which we were able to collect indicated that the thymus was as thoroughly removed in one set of animals as in the other. The long bones of the extremities of the greatly undersized dog No. 8 (Figs. 17 and 18) were slender and perfectly formed, but scarcely two-thirds the length of those of dog No. 8, control. The skull of dog No. 8 was normally proportioned, differing from the skull of the control animal only in respect to size. The differences between the bones of the other three undersized dogs and their control mates lay in size only (Figs. 19, 20 and 21), and were much less marked than those

172. See "Influence of Thymectomy on Growth and Development," p. 444.

which existed between dogs Nos. 8 and 8, control. The skeleton of thymectomized dog No. 37, much larger than No. 37, control, showed no peculiarity as compared with the skeleton of the latter. The bones of dog No. 70 were almost twice as long as those of dog No. 70, control, and proportionately thicker. It will be recollected, however, that the differences in growth which existed between dogs Nos. 70 and 70, control, we were obliged to discount because of the probability that the growth of the control was interfered with by disease. Aside from the exceptions just cited there were no essential differences to be noted in the sizes of the bones of the thymectomized and control dogs. None of the thymusless animals showed any bowing of the shaft of the bones of the legs as determined by postmortem or roentgen-ray examination and none of them showed any pathologic thickening, with the possible exception of dogs Nos. 39 and 40, which were thought, perhaps, to exhibit slight thickening of the femurs as compared with the control animals (determined by roentgen ray) during a short period of confinement in a cage (see protocol of experiment).

Before proceeding further it may be well to point out that the methods which have been used to determine the influence of thymectomy on the growth of the epiphyseal cartilage and on the development of the centers of ossification may be subject to certain inaccuracies or have certain limitations which are not generally appreciated. If sections taken in series through the epiphyso-diaphyseal junction of the end of a long bone—for example, the lower end of the femur—are examined, it is frequently found that the depth and configuration of the cartilage is different in different parts, and, if growth in the bone is drawing to an end, the cartilage is absorbed in some places earlier than in others, in other words, that closure of the epiphysis does not take place in all parts at the same time. It thus becomes evident that before it is possible to infer from sections taken through the lower end of the femur or any other large bone that growth in the epiphyseal cartilage is at a different stage in one animal than in another, it is necessary to know that the sections on which judgment is to be based have been taken from identical places and in exactly the same plane. Basch's observation that the line of junction of diaphysis and epiphysis of the lower end of the femur was more wavy in the thymectomized than in the control dogs of his experiments had no pathologic significance; it meant, in all probability, merely that the sections happened to be taken in the thymectomized dogs nearer the periphery, i. e., through the condyle where the line of junction of epiphysis and shaft normally forms undulations, and in the control animals nearer the middle where the line or junction is almost straight. Basch, apparently, sought to compare sections which were not homologous and, therefore, not comparable, and drew distinctions which did not exist. So, also, it is easy

to be misled from the comparisons of sections into thinking that a certain center of ossification is present in one dog and absent in another, or larger in one animal than in another, for the simple reason that it may have happened that the section passed through the center, in the one instance, and missed it entirely in the other, or cut through the middle in the one case, and in the other through the periphery. Basch's demonstration that the development of the centers of ossification in the control dogs was in advance of that in the thymectomized dogs by means of photographs of sawn, longitudinal sections of the tibias is not absolutely convincing because of lack of assurance that the plane of section in the pairs of bones was identical.

The roentgen ray, on the other hand, offers certain means of determining the relative development of the centers of ossification and time of closure of the epiphyses. It does not indicate, however, the state of development of the epiphyseal cartilage. Knowledge in regard to the latter can be obtained only through microscopic examination. But, microscopic examinaton is an uncertain means of gauging differences in the rate at which growth is taking place because of the difficulty of obtaining exactly homologous sections, on the one hand, and the indefinite character of the criteria on which it is necessary to rely, on the other (depth of the proliferative zone of cartilage, character of the cartilage cells, character of the calcification, evidences of invasion of the cartilage, the relation of new bone formation along the columns of calcified intercellular substance to the cartilaginous border, etc.).

Roentgen-ray examination of fore and hind legs of the thymectomized and control dogs of our experiments, made at irregular and infrequent intervals, and with the object of determining the presence or absence of rachitic changes rather than of differences in development, did not bring to light in the thymectomized animals any peculiarities in the time of appearance or in the rate of growth of the centers of ossification.[173] The comparative microscopic study of the epiphysodiaphyseal junction of the lower end of the femur was made, however, in almost all the thymectomized dogs and their control animals, and was productive of almost constant results. In the femur of only one thymectomized dog, the undersized dog No. 8, were there indubitable signs of retarded development. The lower femoral epiphyses of dog No. 8, control, when examined nine and one half months after operation, were found to have completely fused and scarcely any remnants of calcified cartilage could be found. In the lower end of the femur of thymectomized dog No. 8, on the contrary, a thin band of epiphyseal cartilage, separating the ossified portion of epiphysis from the shaft,

173. The bones were not sectioned specially with a view to the discovery of differences in the relative developments of the centers of ossification.

was clearly visible in both sections through the condyle and in those nearer the middle of the bone (Fgs. 25 and 26). On microscopic examination this band of cartilage was seen to be composed almost entirely of columns of cartilage cells which extended nearly to the epiphyseal side. Calcification of the diaphyseal border of the cartilage was everywhere present, and limited in most places to the intercellular substance between the columns, i. e., it was not present in the intercellular substance between the cells of the columns, which is the case when growth of the cartilage has virtually ceased. The marrow at several points in each of the sections studied was invading the cartilage. Dogs Nos. 39 and 67, however, which, it will be recalled, were also smaller than their control mates, showed exactly the same conditions as the latter in the lower femoral epiphysis; in dogs Nos. 39 and 30, control, the epiphyses were completely closed and all calcified cartilage had disappeared; in both dogs Nos. 67 and 67, control, bands of cartilage of about equal thickness and almost identical arrangement were present. Unfortunately, we were unable to determine whether or not osteochondral ossification in the only remaining undersized dog of our series, dog No. 30, was retarded, because it so happened that the femur of the control of this dog was not saved. Fusion of the lower epiphysis of the femur with the diaphysis had already taken place in dog No. 30, however, although sufficient calcified substance still remained to form a blue line in the stained specimen visible to the naked eye. In dog No. 37 and his larger thymectomized mate the lower femoral epiphysis had closed completely. In all the other members of our series osteochondral ossification in the femur and the other bones examined appeared to be either at the same stage of development in each pair of animals, or else had ceased altogether.

Thus, a single one of our experiments (Exp. No. 7) might be construed to indicate that destruction of thymus function increased the growth of the skeleton; four of our experiments (Exps. 4, 6, 8 and 12) that it decreased the growth of the skeleton; and not one that it accelerated growth, and none that it influenced the time of appearance or rate of growth of the centers of ossification. It is necessary to point out, however, that we do not regard as in any way conclusive our evidence concerning the effect of removal of the thymus on the development of the centers of ossification or growth of the epiphyseal cartilage. Larger series of roentgenograms of the extremities of thymectomized and control dogs than we possess, and sections through a number of different epiphyso-diaphyseal junctions from each animal, would be required for comparison before it would be possible to conclude that the development of the skeletons of our thymectomized and control animals in these respects were, with the single exception already noted (dogs Nos. 8 and 8, control), the same. We have no means of

being certain, for example, that if dog No. 39 had been killed eight or nine months instead of thirteen months after operation signs of retardation of growth in the lower femoral epiphysis might not have been present.

For the delayed closure of the lower femoral epiphysis of dog No. 8, as well as for the extreme undersize of that animal, we have no satisfactory explanation, and cannot be certain that both were not dependent on deprivation of thymus function. It was an interesting and, perhaps, significant fact that growth still should have been in progress in the skeleton of dog No. 8 at a time when it had altogether ceased in dog No. 8, control. We wish it were possible to know to what extent growth in dog No. 8 would have continued and how much of the differences in size and weight which existed between him and dog No. 8, control, would have been reduced had both animals remained alive. The possibility must be admitted that the undersize of dog No. 8 was in the nature of a true *retardation* of growth, and that it would not have remained permanent. If all our thymectomized dogs had behaved like dog No. 8 we should not have hesitated to infer that deprivation of thymus function retarded growth and development of the skeleton in the dog. Considering our experiments in their entirety, however, we believe that it is necessary to conclude, as we did in regard to the influence of thymectomy on the growth and development of the animal as a whole, that deprivation of thymus function certainly does not accelerate growth and development of the skeleton, and that it probably exerts no influence on either.

Though our experiments did not yield data which were absolutely uniform in regard to the effects of removal of the thymus on normal growth and development of the skeleton, yet they yielded perfectly definite information in regard to the question of pathologic changes in the bone of thymoprivic origin and permit us to state with absolute certainty that removal of the thymus gland in the dog does not cause rickets or any allied condition. Disease can be conceived of as causing rickets to develop in the dog but not as preventing its development. If the diseased thymectomized dogs of our experiments had shown rickets, we should have been in doubt as to its origin, and should have been obliged to regard the animals themselves as worthless experimental subjects. When, however, they did not show rickets, they became as valuable for the solution of this particular question as if they had been healthy from the beginning.[174]

174. For this reason we stated that it so happened that all of our thymectomized dogs, whether diseased or not, were available for the study of the question of pathologic developments of the skeleton caused by loss of thymus function.

Schmorl[148] has pointed out that the earliest signs of rickets occur in the middle tier of ribs, because growth of bone is there the most rapid, and that, if active rickets is present anywhere in the body, evidences of the disease will be present in that part of the skeleton. Accordingly, we examined the ribs for rickets in all the thymectomized and control dogs with the exception of one pair; in most of the animals the lower end of the femur also, and in certain of them the tibia, humerus and lower end of the radius in addition. In the one pair of animals we studied only the upper end of the femur. The criteria of rickets which we kept before us were the characteristic changes at the epiphyso-diaphyseal border (notably failure in calcium deposition, invasion of the cartilage with elements from the marrow and cartilage canals), and the development of pathologic borders of osteoid along the trabeculae of the shaft. If rickets is active in a growing bone, changes at the junction of shaft and cartilage, and the development of osteoid borders along the trabeculae, will be coincident. If the rachitic process has recently receded or stopped altogether, the trabeculae, which recover first, may lose their osteoid borders and become normal, but the signs of rickets will persist at the cartilage-shaft border. If osteochondral growth of the bone has ceased, the osteoid border along the trabeculae becomes the sole criterion of the presence of the disease (osteomalacia). Only incipient rickets could possibly be overlooked.

The cartilage of the rib in all of the thymectomized dogs of our experiments was normal; its arrangement was orderly; the cells were in parallel columns. The morphology of the cells was normal. The line of junction between cartilage and shaft was clear cut, and calcium deposition in the cartilage absolutely regular in all but one instance, dog No. 67, Exp. No. 12, in which there were slight changes presently to be described in detail. The osteoid border of the trabeculae, which is considerably wider in the dog than in man, was not in excess of the normal in any one of the thymectomized dogs as determined by comparison with the osteoid border in the control animals. The femurs and the other bones examined did not show deviations from the normal in the direction of rickets or osteomalacia in a single instance. The larger bones of the control dogs, also, were entirely free from any suggestion of rickets or osteomalacia. The ribs of one of the control dogs, No. 71-75, control, did present, however, a pathologic condition of a rachitic nature.

The rib of the control dog in question (No. 71-75, control) (Figs. 29, 30, 31 and 32) was soft, and could be cut with a razor as easily as if it had been composed of soft wood. The junction of cartilage and shaft formed a slightly irregular line marked by tiny serrations visible to the naked eye. As the shaft was approached in the exam-

ination of the stained specimen, the cartilage cells became arranged in a disorderly manner in irregular groups, instead of in parallel columns. Calcium deposition was present in the cartilage adjacent to the shaft, but very irregular. In some places it was absent altogether, in some fragmentary, and in others appeared to be normal. At a number of points there were irregular excavations in the cartilage border into which prolongations from the bone marrow could be seen to extend, making cartilage and shaft appear to interdigitate. The prolongations of the bone marrow were composed chiefly of blood vessels and connective tissue, intermingled with which was much degenerated cartilage. The invasion of the cartilage seemed to be taking place at points which, for the most part, were not protected by calcium deposition. The cartilage which lay adjacent to the shaft failed to stain with hematoxylin at many points, having a light pink color, in contrast to the intense blue of the normal cartilage. In one place this degenerated cartilage appeared to be undergoing metaplasia into bone. A large island of cartilage, showing well marked signs of degeneration, was present in the shaft at some distance from the costochondral junction. In the shaft the trabeculae were long, thin and parallel to each other. Signs of new bone formation along the trabeculae were abundant and osteoblasts appeared to be as numerous as under normal conditions. In many places, however, osteoid, instead of bone, was present. The borders of osteoid were most marked in the shaft at some distance from the cartilage, where the trabeculae were broadest, and reached a width at some points such as is seen only in advanced rickets or osteomalacia in human beings. But the distribution of the osteoid border was not universal. Whereas some trabeculae showed it on two sides, others did not show it at all, or only on one side, or only at one point along the side, or at the tip of a trabeculum. Numerous signs of rarefaction were present. The marrow appeared fairly normal. The femur of this dog, in contrast to the rib, showed no abnormality. The line of junction of cartilage and shaft was clear cut; the calcium deposition in the cartilage was normal. The cells of the cartilage were in parallel columns and had a normal appearance. An osteoid border along the trabeculae of the shaft was only here and there visible and did not exceed the normal. Osteoclasts were present in large numbers.

The rib of thymectomized dog No. 67, already referred to as abnormal (Fig. 33), cut with little resistance. The junction of cartilage and shaft formed, however, approximately a straight line; the width of the cortex appeared to be normal. Near the shaft the cartilage cells were arranged in parallel columns and had a normal appearance. Deposition of calcium in the cartilage was, however, irregular. In several places, for distances equal to the width of three to seven cartilage columns, calcium deposition was wanting; elsewhere it appeared to be

essentially normal. Marrow vessels invading the cartilage columns were slightly in advance of the rest at those places in which calcium deposition was wanting, so that the line of junction of cartilage and shaft, as seen under the microscope, was not perfectly even. The columns of calcified intercellular substance, adjacent to the cartilage, were numerous, parallel and showed beginning bone formation along their sides. A short distance away from the cartilage border, a distinctly pathologic condition presented itself in the form of a calcium free zone, not more than from two to three cartilage cells wide, which traversed the shaft at right angles, more or less parallel to the line of junction of cartilage and shaft. This bandlike zone did not extend continuously from one side of the shaft to the other, however, for at two or three points normal calcified columns of intercellular substance interrupted it. Where continuity of the columns of calcified intercellular substance was broken by the bandlike zone, it was found in most places that small masses of cartilage cells having more or less of a columnar arrangement and lying in the line of the calcified columns, filled in the gaps between. These groups of cartilage cells were not only free from calcium deposition, but had the appearance of being compressed and showed signs of degeneration. They stained pink with eosin. Farther down in the shaft, osteoid borders were visible here and there along some of the trabeculae. Although not so broad or so numerous as those seen in the rib of the control dog No. 71-75, control, they were, in places, exceedingly well developed. They occurred, with a few exceptions, only in the thicker trabeculae and quite uniformly on one side or at a single point along one side. Many of the larger trabeculae did not show any border of osteoid. Apparently, considerable rarefaction was present. The marrow cells were greatly diminished in number. The other representatives of the middle tier of ribs showed, with some modification, the condition which has just been described. The lower end of the femur, the upper end of the tibia, upper end of humerus and lower end of radius of the dog proved, however, to be normal; they showed no disturbance in calcification of the cartilage; the osteoid border did not appear wider than normal (Figs. 34 and 35).

The narrow, calcium free zone, which, with some interruption, extended almost across the shaft in the ribs of dog No. 67, could be interpreted only on the ground that, for a comparatively brief period (the time required for the proliferation of from four to five cartilage cells), shortly before the animal was sacrificed, calcium deposition in the proliferative cartilage had suddenly been almost completely arrested. The irregularities in the calcification of the proliferative cartilage indicated further that a disturbance in calcium deposition was existent at the time when the animal was sacrificed.

The extremely defective deposition of calcium in the proliferative cartilage of the ribs of dogs No. 71-75, control, the invasion of the cartilage at those points in which calcium deposition was deficient with elements from the marrow, and the resulting irregularities at the cartilage shaft junction, the wide osteoid border along some of the trabeculae were highly suggestive of rickets. We could not be certain, however, that the pathologic condition in the ribs of dog No. 71-75, control, and dog No. 67 was actually rickets. The condition at the cartilage and shaft junction of the ribs of dogs No. 71-75, control, almost duplicated the condition found in the ribs of human beings with incipient rickets, but did not correspond to it in all particulars; at least, the calcium deposition in those parts of the proliferative cartilage which were not invaded by the marrow elements was more abundant than is usually found in corresponding areas of the cartilage in human rickets. Moreover, the osteoid border in the shaft was not so uniformly distributed as is commonly the case in the rickets of human beings. The condition at the cartilage shaft border in the ribs of thymectomized dog No. 67 bore little resemblance to the early rachitis of human beings. The line of junction of shaft and cartilage was comparatively straight. Invasion of the cartilage with marrow elements, though perhaps about to take place, had not actually occurred. The osteoid borders in the shaft were less well-developed and less uniform than in the rib of dogs No. 71-75, control. We do not know how closely the rickets of animals resembles the rickets of human beings. It is clear, however, that, if the disturbance in the process of ossification in dogs Nos. 67 and 71-75, control, was not rickets, it was a closely related condition, which had its basis, like rickets, in a disturbance in the normal process of calcification. Whether or not the condition in the bones of dogs Nos. 71-75, control, and 67 was rickets is actually immaterial. The presence of the condition arising spontaneously in the control animal negates the significance of its occurrence in the thymectomized animal.

Though none of our dogs, with the two possible exceptions noted, showed any rachitic lesion, a large number of them, control and thymectomized dogs alike, exhibited varying degrees of osteoporosis (Figs. 36 and 37). All the dogs which had been sick for some time previous to death and had emaciated, whether thymectomized or control animals, showed marked osteoporosis. There was no reason to suppose, therefore, that the osteoporosis was in any way dependent on deprivation of thymus function. We noted with interest that osteoporosis can reduce the resistance of the bone to cutting to an extraordinary degree. Moreover, we found, as there was every reason to anticipate, that osteoporotic bones could be fractured with little force. We call attention to

these facts, merely to point out that great reduction in the tensile strength of a bone and in the resistance which it offers to cutting does not signify that the pathologic condition present is rickets.

We conclude that destruction of thymus function in the dog does not cause rickets or any disease of the skeleton.

THE EFFECT OF REMOVAL OF THE THYMUS ON THE SPLEEN AND ORGANS OF INTERNAL SECRETION

The endocrine glands and the spleens of all the thymectomized and control animals of our experiments were examined microscopically, except in a few instances in which it so happened the organs were not saved. Some of the endocrine glands from certain animals, control as well as operated, proved to be of no value, however, for the study of the effects of extirpation of the thymus on account of the presence in them of extensive pathologic changes, which it was certain were not connected in any way with deprivation of thymus function. The suprarenal, thyroid, pancreatic glands and testes of several animals not necropsied immediately showed widespread degenerative alterations of postmortem origin, and the thyroids of a number of animals acutely sick when sacrificed presented such extensive exfoliation and swelling of the alveolar epithelium as to destroy all traces of any antecedent condition. The parathyroid and pituitary glands, in contrast to the others, were found to be uniformly well preserved.

We were unable to discover any alterations in the spleens of the thymectomized dogs of our experiments which were not to be found in the spleens of the control dogs also. In some pairs of animals the malpighian bodies appeared to be somewhat larger in the thymectomized animal, in other pairs in the control animal, and in still other pairs they appeared to be of about equal size in both. As a matter of fact, we found it extremely difficult to make comparisons in regard to the relative size or number of the malpighian bodies in the spleen of the thymectomized and control animals. On microscopic examination of the spleen we could not find evidences of hyperplasia of the malpighian bodies in the thymectomized animal. Consequently, we were wholly unable to confirm the findings of hyperplasia of the malpighian bodies which Klose and Matti reported in their thymectomized animals. We could not detect any abnormalities in the splenic pulp of the thymectomized dogs, and failed to find that any peculiar type of cell, such as the multi-nucleated, giant cell described by Flesch, was in especial abundance in the spleens of the thymectomized animals.

We could not fail to be impressed with the fact that the spleens of those thymectomized and control dogs that were greatly malnourished were in a state of extreme atrophy. They were greatly reduced in

size and weight; on section the cut surface was found to be light red in color and comparatively dry; the malpighian bodies were scarcely visible to the naked eye. On microscopic examination they were seen to consist of small groups of cells, and could be identified only by their relation to an arteriole; the pulp was atrophic and the trabeculae were so numerous and close together as to compose from one third to one half of any given microscopic field. It will be remembered that Klose laid great emphasis on the extreme atrophy of the spleen shown by the thymectomized dogs of his experiments, which died or were sacrificed during the "stage of cachexia," and interpreted it as signifying complete exhaustion of the spleen in its ineffectual effort to carry the burden of thymus function in addition to its own. We were spared, however, the temptation to regard the atrophic condition of the spleen in our emaciated, thymectomized animals as having any specific import because the control dogs which were in a state of malnutrition at the time of death exhibited spleens with the identical changes. Indeed, it was so clearly demonstrated in our experiments that splenic atrophy accompanies atrophy of the animal as a whole that we cannot refrain from expressing astonishment that Klose should have journeyed so far into the realms of fancy for an explanation of the atrophic condition of the spleen in the cachectic, thymectomized dogs of his experiments.

The comparison of the weights of the spleens of thymectomized and control dogs in our experiments served merely to emphasize the extreme variability of the spleen and its dependence, in respect to size and weight, as well as morphology, on a whole variety of conditions which have no relationship to loss of thymus function.

Not only did extirpation of the thymus produce no change whatsoever in the spleen in the dogs of our experiments, but we doubt whether deprivation of thymus function has been the cause of the changes reported in the spleen in the experiments of other investigators.

We could not find any alterations in the pancreas,[175] hypophysis, parathyroids or adrenals in our thymectomized dogs which we could possibly attribute to deprivation of thymus function. The glandular elements of the pancreas certainly failed to show the slightest variation from the normal. Whether the islands of Langerhans were more numerous or less numerous in the thymectomized dogs than in the control animals, we did not attempt to determine. They appeared to be normal in structure and arrangement, however, in both sets of animals. The pancreatic glands of two thymectomized animals showed lesions which were clearly of an inflammatory nature and not connected in any way with deprivation of thymus function.

175. The weight of the pancreas was not recorded in many of our experiments.

Comparative study of sections from the hypophyses of the thymectomized and control dogs of our experiments was not entirely satisfactory, in the first place, because the sections were not complete in a number of pairs of animals and, in the second place, because in scarcely a single pair were the sections taken through exactly the same parts of the gland. The sections of the hypophyses of the thymectomized animals studied showed, however, no abnormality, not even the sections from those animals some of whose other organs showed marked degenerative or inflammatory changes.

The parathyroids were examined microscopically in the majority of thymectomized and control dogs and were found to be normal in all but a few animals.[176] In these sclerosis of the parathyroid, in varying degree, was present.

We were wholly unable to confirm Matti's statement that removal of the thymus is followed by hypertrophy of the medulla of the suprarenal. Although there appeared to be variations in the width of the medulla of the suprarenal in some pairs of dogs, they were not extreme and not constant. It was certainly impossible to say that the medulla of the suprarenal was larger in one set of animals than in the other.

As a matter of fact, there is need of great caution before concluding that the medulla of one suprarenal is larger than the medulla of another for the simple reason that it is impossible to determine the size of the medulla by direct measurement. The only method by which the size can be estimated accurately is an indirect one and involves complete serial section of the gland and reconstruction of the medulla from the sections. That method, however, has never been employed by those who have reported medullary hypertrophy after thymectomy, so far as we are aware. Klose and Matti came to their conclusions, we are led to suppose, by comparison of cross sections. It is scarcely necessary to point out, however, how difficult it is to be certain that the medulla of one suprarenal is actually larger than the medulla of another from a comparison of cross sections, particularly if the latter are taken from the glands in question at random. In the first place, it is difficult to measure the length of the medulla with any degree of accuracy. In the second place, it is difficult always to be sure that the sections are at right angles to the long axis of the gland. If they chance to be oblique, it is obvious that the width of the medulla would appear to be greater than it actually is. In the third place, the normal variations in both breadth and thickness of the medulla in different parts of the gland is so great that differences in the size of the medulla in any pair of homologous sections has only a local significance, and differences in sections which are not homologous have no significance

176. Both thymectomized and control animals.

whatsoever. Finally, prolongations of the medulla extend outward between folds of the cortex, which cannot be taken into consideration short of complete serial section. We do not wish to imply that it is impossible ever to infer correctly that the medulla of one suprarenal is larger than the medulla of another from the examination of cross sections, for if the differences are sufficiently great, i. e., if the medulla of one suprarenal chances to be two or three times the size of the medulla of the other suprarenal, there can be no mistaking the fact. We wish to make clear, however, that if the differences in size of the medullae of two suprarenals are not extreme, it is easy to be misled, even when the comparison of sections is conducted in the most systematic manner, and that a judgment based on the comparison of only one or two pairs of sections, or of sections taken at random from the two glands, is of no real value.

We should not have taken such pains to point out the difficulties in the way of reaching positive conclusions in regard to the size of the medulla of the suprarenal except for Klose's casual remark that "the medulla of the suprarenal undergoes hypertrophy without exception after extirpation of the thymus . . ."[177] and for photographs of sections from the suprarenals of two thymectomized dogs and one control dog, published by Matti to illustrate the differences which existed in the size of the medulla in the suprarenal of the thymectomized and control animals of his experiments. If Figure 38, reproduced from Matti's publication, is examined, it will be seen that the section of the suprarenal taken from the control dog is long and thin and the medulla scarcely more than a line in thickness, but that the sections of the suprarenals from the two thymectomized dogs are much shorter and are broad and show medullae having a more or less oval outline and equalling or exceeding the cortex in thickness. If the suprarenal from a normal dog is examined (Fig. 39), it is often found to be broad and flat in one part, but to have a more or less cylindrical form in another. If a section is taken through the broad and flat portion, it will be found to be shaped very much like the section from the suprarenal of Matti's control dog and the medulla, as seen in the section, will appear long and exceedingly thin. If, on the other hand, the section is taken through the thicker part of the gland, it will be found to have the shape and appearance of the sections from the suprarenals of Matti's two thymectomized dogs, and the medullae will appear oval or actually round and will be as broad, or twice as broad, as the cortex. In other words, the photographs of sections which Matti published to demonstrate that the medulla of the suprarenal was larger

177. Das Nebennierenmark geht nach Thymusausschaltung, wie zuerst Matti nachweisen konnte, ausnahmslos eine Hypertrophie ein" (p. 328).

in the thymectomized than in the control dogs of the experiments could have been duplicated from different parts of the single suprarenal of many normal animals. Matti's illustrative sections really not only fail to prove his case, but suggest the possibility that he may have based his conclusion that extirpation of the thymus gland causes the medulla of the suprarenal to hypertrophy on the study of sections from different parts of the two glands which were not homologous and, therefore, not comparable.

In regard to the finer histology of the suprarenals, it is possible for us to state only that we noted no essential differences in the two sets of animals. We were not able to detect any histologic evidence of hyperplasia in the medulla of the suprarenals from the thymectomized dogs which did not occur in the control animals also. The architecture of the cortex and the morphology of the cells of the three zones was apparently the same in both thymectomized and control dogs. The lipoid content of the suprarenal of the dogs of the first eight experiments, as roughly indicated by the sudan III method, exhibited marked variations, but not in thymectomized as against control animal.

Although the suprarenal glands of the thymectomized and control dogs showed comparatively slight differences in their finer histology and no great differences in the size of the medullae about which it was possible to be certain, they did show, in some instances, considerable variations in weight. For example, the suprarenal glands of dog No. 13 were 0.026 per cent. of the body weight of the animal, but the suprarenals of one of the two control dogs of the litter to which this animal belonged, dog No. 12, control, were only 0.010 per cent. of the body weight.[178] The weight of the suprarenals of dog No. 8 was 0.019 per cent. of the body weight, but the weight of the suprarenals of dog No. 8, control, was 0.012 per cent. of the body weight. The weight of the suprarenals of dog No. 70 was 0.025 per cent. of the body weight, but the weight of the suprarenals of dog No. 70, control, was 0.038 per cent. of the body weight. Out of a total of seventeen pairs of thymectomized and control dogs, the relative weights of the suprarenals were found to be approximately the same in eight; in seven pairs the relative weight of the suprarenals was greater in the case of the thymectomized animal, and in two pairs in the case of the control animal.

Overmuch importance cannot be placed on these statistical differences, however. In the first place, the weights of the suprarenals of the control animals, themselves, showed marked variation, which cer-

178. The suprarenals of the other control of this litter weighed 0.015 per cent. of the body weight and the suprarenals of the other two thymectomized dogs, Nos. 10 and 12, weighed 0.017 per cent. and 0.015 per cent. of the body weight, respectively.

tainly could not be ascribed to disturbance of thymus function. For example, the suprarenals of control dogs Nos. 71-75, control, were 0.015 per cent. of the body weight, but the suprarenals of control dogs. Nos. 72-73-74, control, of the same litter, were 0.023 per cent. of the body weight. The suprarenals of control dog No. 60 were 0.049 per cent. of the body weight, whereas the suprarenals of control dog No. 61 of the same litter, were 0.035 per cent. of the body weight. In the second place, it must be remembered that a number of the animals (chiefly the thymectomized animals), the weights of whose suprarenals were considered, were sick from causes unrelated to deprivation of thymus function and were greatly emaciated at the time of death. Jackson[179] has found that the thyroid of the albino rat lost but little weight in acute inanition of sufficient degree to cause the body as a whole a loss of one third the total weight, but that in prolonged inanition, the thyroid lost from 22 to 24 per cent. of its weight. We do not know the relation the weight of the suprarenal bears to the body weight in varying nutritional states, but doubt whether it varies directly with that of the body as a whole.

Our experiments as a whole fail to prove that extirpation of the thymus exerts any influence on the suprarenal. It is impossible to be certain, however, that removal of the thymus may not have caused the suprarenals of some of the animals to hypertrophy.

Although it was possible to state with considerable assurance that no changes of a histologic nature were present in the spleen, pancreas, suprarenals, parathyroids and hypophysis of the thymectomized dogs of our experiments which did not occur in the control animals also, it was not so easy to be certain that no changes of thymoprivic origin were present in the thyroids of the thymectomized dogs. One reason for our uncertainty in regard to the thyroids of the thymectomized animals was that the thyroids of the control animals showed the widest possible range of variation, failing to furnish any common type which could be used as a standard of comparison for the thyroids of the operated animals, and making it necessary to regard the great differences in the structure of the thyroid glands of the operated dogs as a normal condition. The thyroid of dog No. 7, control, for example, (Fig. 40) showed a typical colloid picture with large, strikingly regular acini lined with flat epithelium and showing well marked atrophy of the wall. The thyroid of dog No. 1, control, on the other hand, was characterized by the presence of a moderate amount of colloid, high cuboidal or cylindrical epithelium which appeared rich in chromatin

179. Jackson, C. M.: Effect of Acute and Chronic Inanition on the Relative Weights of the Various Organs and Systems of Adult Albino Rats, Anat. Rec. **9**:90, 1915.

and by beginning infoldings of the acinar walls. As a matter of fact, the thyroids of the operated dogs did present fully as great, if not even greater, variations in structure than the thyroids of the control animals. For example, the thyroid of thymectomized dog No. 7 (Fig. 41) contained masses of colloid in large alveoli with thinned out walls and flat epithelium. In sharp contrast, however, the thyroid of dog No. 8 contained little colloid—in some places almost none—and though the structure of the gland in some parts was normal, in other parts it exhibited well marked signs of hyperplasia. When, however, differences could be found in the thyroids of a thymectomized and control animal, the variation in the thyroid of the thymectomized animal from the standard set by the thyroid of the control animal, was generally on the side of hyperplasia. Although the thyroid of dog No. 7, for example, was as well marked a colloid type of gland as could be found, yet the alveoli were not uniformly so large as those of dog No. 7, control. Although the thyroid of dog No. 8 presented evidences of focal hyperplasia and contained a diminished amount of colloid, the thyroid of dog No. 8, control, was a simple colloid gland (Figs. 42 and 43). In almost all the pairs of thymectomized and control animals, however, the differences in the thyroids were comparatively slight and warranted the conclusion that, if the removal of the thymus had exerted any influence, it could not have been very great, at least it had not been sufficient to overcome environmental influences or hereditary tendencies—in other words, to modify to any great extent what must be considered normal variation.

MacLennan[83] was the first to report histologic changes in the thyroid in the nature of hyperplasia following thymectomy and his discoveries have been confirmed by Matti[4] and by Klose[92] in their investigations.[180] Adler[49] also has reported changes in the thyroid of a hyperplastic character in the thymectomized tadpole (three experiments). Pappenheimer,[133] who studied the thyroid of the thymectomized rat with especial care, was not able to detect any changes which were constant. Other investigators, also, have failed to note changes in the thyroid after thymectomy.

In this connection it may not be amiss to point out that the thyroid is the one of the endocrine glands which preeminently exhibits marked, unaccountable variations from the normal type and

180. It should be pointed out, however, that Matti's and Klose's corroboration of MacLennan's findings of hyperplasia in the thyroid of thymectomized animals has not quite the force which it might have had, if their findings and MacLennan's findings, in almost all other respects, had not been directly the opposite. MacLennan came to the conclusion that thymectomy stimulated growth and that its effect on the animal was "beneficial," whereas Klose and Matti found that it causes dwarfing and finally death.

also responds with well marked structural alterations to a variety of environmental influences, some of which appear to be exceedingly mild. A number of investigators recently have called attention to these peculiarities or special properties of the thyroid in several different species of animals. For example, Hunnicutt[181] has pointed out the great variability in the histologic structure of the thyroid in the dog. Sections taken from the thyroids of dogs that were apparently normal showed marked hyperplasia in 32 per cent. and early signs of hyperplasia in 5 per cent. Douglas[182] examined the thyroids of pigeons, fowls, rats and rabbits to determine the extent of normal variation in the histologic structure of the thyroid, and concluded that the variation in different animals of the same species kept under the same conditions was so extreme that an interpretation of experimental results, based on histologic alterations in the thyroid, was a matter of extreme difficulty. Fox[183] reported marked variations in the thyroids of wild animals in confinement. McCarrison[184] made comparative studies of the thyroid gland of wild rats, white rats and mice kept in confinement and goitrous rats, both wild and domesticated. It was his observation that goiter was exceedingly rare but that the structure of the thyroid gland varied in wild rats within wide limits and appeared to be especially influenced by altitude and season. The thyroid was found to be in a state of active secretion much more frequently in the domesticated white rat than in the wild rat. Goiter developed spontaneously in a small proportion of white rats confined in cages. Jackson[179] investigated the effects of starvation on the thyroid. He found that when young rats were kept on a diet just sufficient for maintenance for several weeks, changes made their appearance in the thyroid; the follicular epithelium became atrophied and reduced in height, the nuclei hypochromatic and pyknotic and, in extreme cases, fragmented; the cytoplasm was more affected than the nucleus; the colloid appeared normal in some glands, in others disintegrated or replaced by desquamated epithelial cells. Jackson thought that the changes just enumerated were in no wise specific but such as are encountered in the thyroid under a variety of pathologic conditions. Bensley,[185] also,

181. Hunnicutt, J. A.: The Absence of Hyperplasia of the Remainder of the Thyroid in Dogs after Piece-Meal Removal of This Gland; Auto-Transplantation of the Thyroid in Partially Thyreoidectomized Animals, Am. J. M. Sc. **148**:207, 1914.

182. Douglas, M.: The Histology of the Thyroid in Animals fed on Various Diets, J. Path. & Bacteriol. **19**:341, 1914.

183. Fox, H.: The Pathology of the Thyroid Gland in Wild Animals, Proc. Path. Soc. Phila. **17**:20, 1915.

184. McCarrison, R.: Nervous Cretinism, Brit. J. Dis. Child. **11**:508, 1914.

185. Bensley, R. R.: The Normal Mode of Secretion in the Thyroid Gland, Am. J. Anat. **19**:37, 1916.

has commented on the variability of the structure of the thyroid in animals and the susceptibility of the thyroid to environmental influences.

"The readiness with which the thyroid gland undergoes hyperplastic changes, its responsiveness to iodin administration, as demonstrated by Marine and his co-workers, the ease by which it may be modified structurally by dietary condition, as shown by the work of Reid Hunt (1911), Marine, Chalmers Watson (1907), Tanberg (1900) and Missiroli (1910), and confirmed by my recent studies on the relation of diet to hyperplasia in opossums under domestication, confirm the conclusion that there is a delicate adjustment between the functioning of the thyroid gland and general body conditions, though at present we do not know the means by which this adjustment is mediated." [186]

We have already commented on the wide range of variation displayed by the thyroid of the control dogs of our experiments.

The thyroids of our thymectomized and control dogs were, on the whole, remarkably constant in weight, and exhibited as slight variation in that regard as any other organ. It is certain that extirpation of the thymus did not affect the weight of the thyroid of the dogs of our experiments.

In view of the great variability of structure which characterizes the thyroid, attested to by so many observers, and in view of the tendency exhibited by the gland to undergo changes as the result of influences which are not easy of recognition or analysis, we believe the following conditions must be fulfilled before it is possible to consider that variations from the normal type in the thyroids of thymectomized animals had their origin in loss of thymus function:

1. The number of thymectomized animals whose thyroid glands are examined must be sufficient to exclude chance variation as the determining factor.

2. For the thyroid of each thymectomized dog examined, there must be examined a thyroid from a control animal of the same litter.

3. The thyroids of the thymectomized dog must conform to a common type; they must not exhibit, however, the characteristic feature or features of the thyroids of the control animals. In other words, a thymoprivic influence must be strong enough to overcome normal variation, or its existence cannot be accepted.

We conclude, therefore, that our experiments fail to show that destruction of thymus function produces any effect on the thyroid gland. The minor variations in the structure of the thyroid of the thymectomized dogs from the standard established by the thyroids of

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186. Park: Review of Literature of Past Two Years on Organs of Internal Secretion, Am. J. Dis. Child. **12**:477 (Dec.) 1916.

Fig. 28.—Dog 10 (rib)

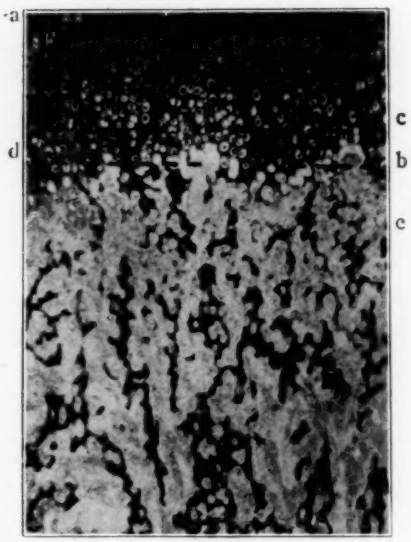

Fig. 29.—Dog 71-75C (rib)

Fig. 28.—Photomicrograph of rib of dog No. 10, Exp. 6. High power magnification. Thymectomy performed on the ninth day. Period of observation, seven months and three days. (a) Cartilage; (b) calcium deposition in the cartilage forming almost a solid band and infiltrating the cells themselves as well as the intercellular substance; (c) trabecula of shaft. The deposition of calcium in the cartilage is characteristic of the condition regularly found in the rib when growth has practically ceased. Decalcified by liquor formaldehydi-formic acid method. Stained with hematoxylin and eosin.

Fig. 29.—Photomicrograph of rib of dog No. 71-75, control, Exp. 14. Low power magnification. Age at death, 2 months and 13 days. (a) Cartilage; (b) calcium deposition in cartilage; (c) areas of cartilage in which calcium deposition has not taken place; (d) marrow elements invading cartilage in places unprotected by calcium deposition; (e) degenerated cartilage extending into shaft. Note the extreme irregularity of cartilage-shaft junction and the disorderly arrangement of the cartilage bordering on the shaft. The condition at the cartilage-shaft border simulates almost exactly the condition in the rib in incipient rickets of human beings. Decalcified in Müller's fluid. Stained with hematoxylin and eosin.

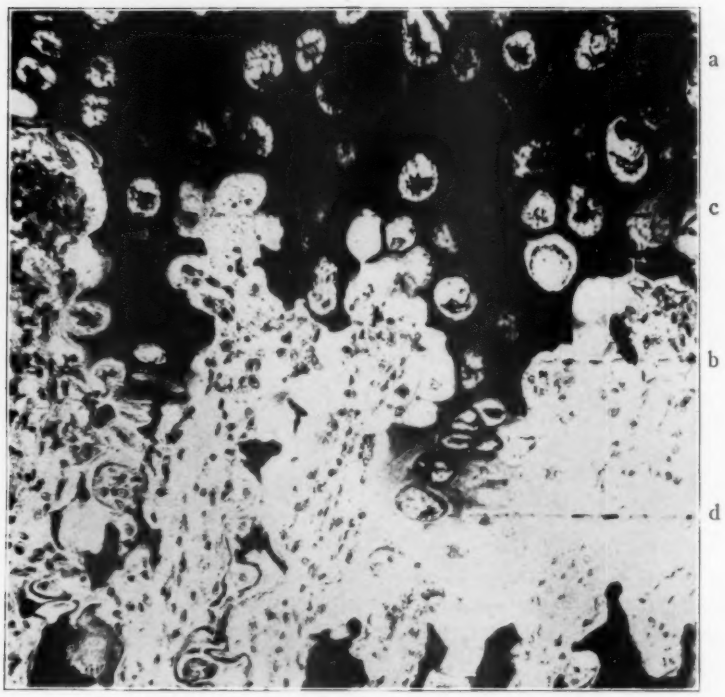

Fig. 30.—Photomicrograph of rib of dog No. 71-75, control. Higher power view. (a) Cartilage; (b) calcified intercellular substance; (c) marrow elements invading cartilage in places unprotected by calcium; (d) degenerated cartilage. Condition almost identical with the condition in the rib in incipient rickets of human beings. Decalcified in Müller's fluid. Stained with hematoxylin and eosin.

Fig. 31.—Photomicrograph of rib of dog No. 71-75, control, showing trabeculae at some distance from costochondral junction. High power magnification. (a) Trabecula; (b) marrow; (c) broad border of osteoid; (d) osteoblasts lining osteoid border. Note the extreme width of the osteoid borders, such as is seen only in advanced rickets in human beings. Decalcified in Müller's fluid. Stained with hematoxylin and eosin.

Fig. 32.—Photomicrograph of rib of dog No. 71-75, control, showing higher power view of osteoid border, in order to demonstrate its extreme width. (a) Trabecula; (b) marrow; (c) broad osteoid border along one side of trabecula; (d) osteoblasts lining osteoid border. Decalcified in Müller's fluid. Stained with hematoxylin and eosin.

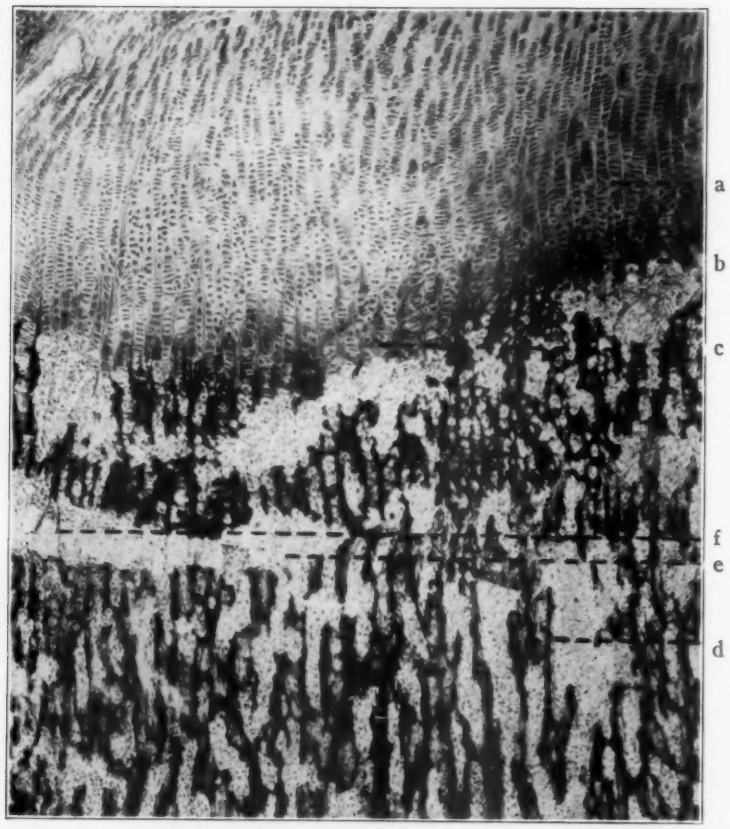

Fig. 33. Photomicrograph of rib of thymectomized dog No. 67, Exp. 12; showing junction of cartilage and shaft. Photographed when 3 months and 4 days old. Period of observation, two months and nineteen days. Low power magnification. (a) Proliferative cartilage arranged normally in parallel columns; (b) calcium deposition in cartilage; (c) areas of cartilage in which calcium deposition is wanting; (d) trabeculae; (e) calcium free band extending transversely across shaft parallel to cartilage and shaft junction; (f) cartilage cell columns containing no calcium in the calcium free band. Evidently a marked disturbance in the calcification of the cartilage has taken place. In that respect the condition simulates rickets. Invasion of the cartilage with narrow elements in the areas unprotected by calcium has not occurred. Except for the irregularities in the calcium deposition in the cartilage, the condition is not like rickets as it occurs in human beings. Decalcified in Müller's fluid. Stained with hematoxylin and eosin.

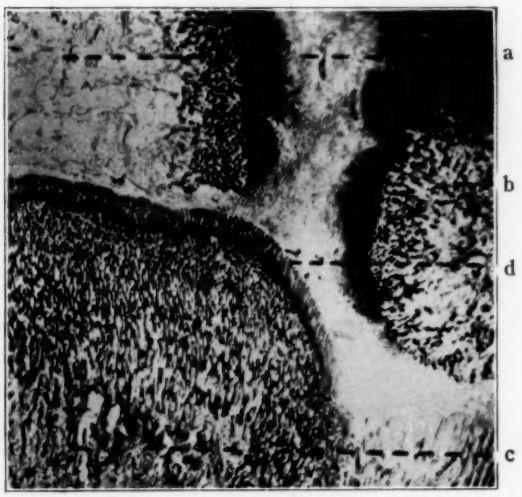

Fig. 34. Photomicrograph of tibia of thymectomized dog No. 67, showing upper epiphysis and nucleus of ossification of tuberosity. Low power magnification. (a) Epiphysis; (b) nucleus of ossification of tuberosity; (c) shaft; (d) black line of calcium deposition. No evidence of rickets. Decalcified in Müller's fluid. Stained with hematoxylin and eosin.

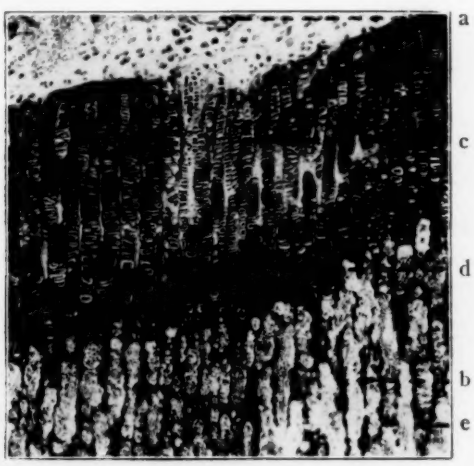

Fig. 35. Photomicrograph of dog No. 67, showing junction of upper epiphysis of tibia with shaft. Low power magnification. (a) Epiphysis; (b) diaphysis; (c) proliferative cartilage arranged in parallel columns; (d) zone of calcium deposition in cartilage; (e) trabeculae. Note that conditions in tibia are absolutely normal in contrast to conditions in rib (Fig. 25). There is absolutely no evidence of any disturbance in the calcification of the cartilage in the tibia. Decalcified in Müller's fluid. Stained with hematoxylin and eosin.

Fig. 36.—Dog 10 (femur)

Fig. 37.—Dog 30 (rib)

Fig. 36. Photomicrograph of femur of thymectomized dog No. 10, Exp. 5. Low power magnification. Thymectomy performed on the ninth day. Period of observation, seven months and three days. (a) Epiphysis; (b) proliferative cartilage; (c) line of calcium deposition in proliferative cartilage; (d) trabeculae. Note the extreme degree of osteoporosis. No sign of rickets. Decalcified by liquor formaldihydi-formic acid method. Stained with hematoxylin and eosin.

Fig. 37. Photomicrograph of rib of thymectomized dog No. 30, Exp. 6. Low power of magnification. Period of observation, ten months and twenty-one days. Age at operation, 6 weeks. (a) Proliferative cartilage; (b) zone of calcium deposition in cartilage; (c) trabeculae of shaft showing rarefaction. No sign of rickets. Decalcified by liquor formaldehydi-formic acid method. Stained with hematoxylin and eosin.

Figure 38

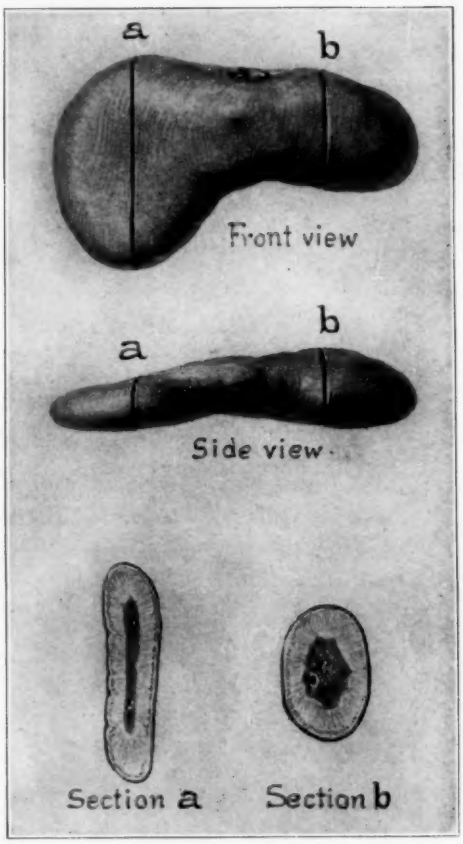

Figure 39

Fig. 38. Reproduced from Matti's article (Ergeb. d. inn. med. u. Kinderh.). A sketch of sections through the suprarenals of two thymectomized and one control dog, designed to demonstrate that removal of the thymus causes the medulla of the suprarenal to hypertrophy.

Fig. 39. Drawing of suprarenal and its cross sections to show that cross sections exactly like those shown by Matti can frequently be obtained from different parts of a single suprarenal. In that part of the suprarenal which is broad and flat, the medulla is almost invariably found, on cross section, to be long and narrow, as in Fig. 39. In those parts which have, on cross section, a more rounded or oval shape, the medulla is usually found to be oval, or actually round and to be as broad or even twice as broad as the cortex. The difference in the cross sections published by Matti in reality means nothing.

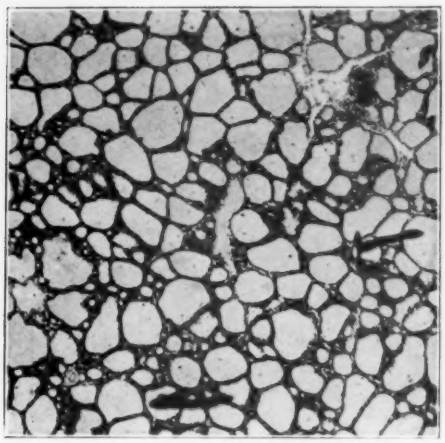

Fig. 40.—Dog 7 Control (thyroid)

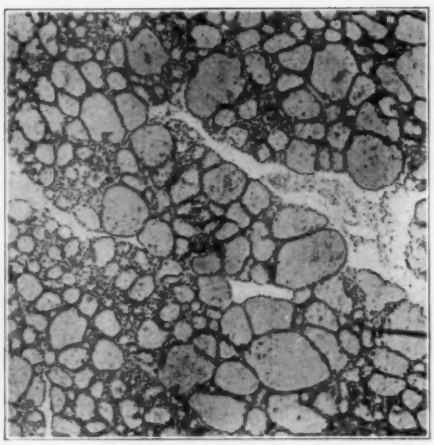

Fig. 41.—Dog 7 (thyroid)

Fig. 40. Photomicrograph of thyroid of dog No. 7, control. Magnified fifty-five times. Note that the alveoli are on the average even larger than those of thymectomized dog No. 7 and are filled with colloid.

Fig. 41. Photomicrograph of thyroid of thymectomized dog No. 7, Exp. 3. Magnified fifty-five times. Note the large size of the alveoli, the thinness of the alveolar walls and the great abundance of colloid.

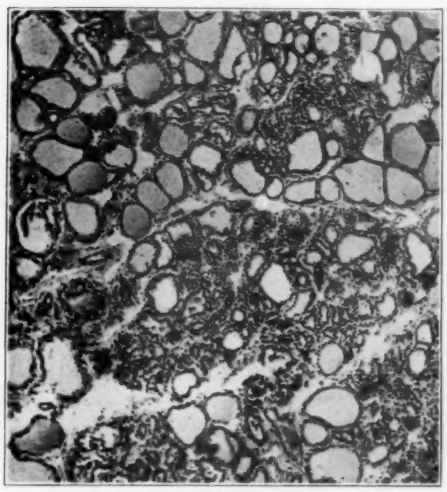

Fig. 42.—Dog 8 (thyroid)

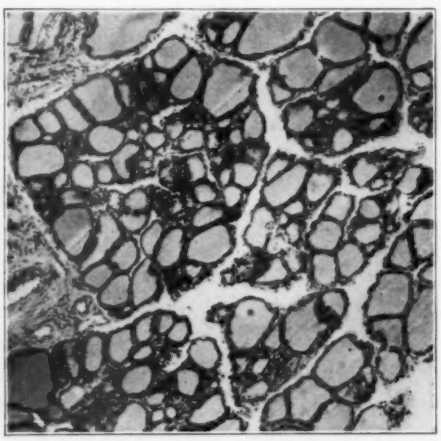

Fig. 43.—Dog 8 Control (thyroid)

Fig. 42. Photomicrograph of thyroid of dog No. 8, Exp. 4. Note the extreme variation in the size of the alveoli. The large alveoli are filled with colloid and the epithelium is low cuboidal. Many parts of the section appear to be greatly condensed and present a solid appearance. In these areas the thyroid acini have almost lost their regular contour and arrangement and there is apparent increase in the parenchymal cells without formation of definite acini. The section shows focal hyperplasia. Otherwise a fairly normal looking gland. Magnified fifty-five times.

Fig. 43. Photomicrograph of thyroid of dog No 8, control. Presents a normal appearance in contrast to the thyroid of dog No. 8. Magnified fifty-five times.

their respective control animals serve, at most, to suggest the possibility that extirpation of the thymus may exert an influence on the thyroid.

The testes of the thymectomized dogs showed no variations from the testes of the control animals except in two instances, dogs Nos. 7 and 10. The testes of dog No. 7 weighed only 15.7 gm., whereas the testes of dog No. 7, control, weighed 28 gm. On microscopic examination the testes of dog No. 7 were found to be incompletely developed. Spermatids were present but no spermatozoa either in the testes or in the epididymes. Spermatozoa were present in abundance, however, in the testes and epididymes of control dog No. 7. Dog No. 7 was more than 13 months old and should have had, like his control animal, a fully developed organ. The only reason which we could find for the retardation in the development of dog No. 7, aside, of course, from deprivation of the thymus function, was that he had been more severely affected by the distemper than dog No. 7, control, and was still suffering from it at the time of his death. The testes of dog No. 10 weighed only 2.5 gm. and were in an embryonic stage of development. It must be remembered, however, that dog No. 10 died when a little more than 7 months old, after a sickness of two and one half months' duration, characterized by marked emaciation. Retardation in the development of the sexual organs might easily be explained and, in fact, was to have been expected in the case of dog No. 10, on the ground of disease. The retardation of the growth of the testes in the case of dog No. 7 may, perhaps, have had its explanation in disease, but it is possible that deprivation of thymus function may have been the determining cause. Against the view, however, that destruction of the thymus function was responsible for the underdevelopment of the testes of dog No. 7, stand the results of all of our other experiments on male animals, in which spermatogenesis seemed to be as advanced in the thymectomized as in the control animal. No differences, whatsoever, could be found between the testes of the young thymectomized dogs and their control mates. The description of their organs do not in all instances appear in the protocols.

The ovaries of the thymectomized female dogs showed no essential differences from the ovaries of their control animals.

The liver, kidneys, heart, muscles, stomach and small and large intestines were examined in a number of the thymectomized animals and found to be normal. The stomach was examined with particular care on account of Klose's observation of the complete absence of acid cells in the stomachs of two of his thymectomized dogs. Acid cells were present in the stomachs of all of our thymectomized dogs examined.

We are unable to form any idea whether or not the lymphatic tissue of the body was affected by removal of the thymus on account of the extreme variations which it displayed in both thymectomized and control dogs. We saw no evidence to suppose that extirpation of the thymus altered in any way the follicles in the intestinal tract.

SUMMARY OF EXPERIMENTAL RESULTS

The results of our experiments may be summarized as follows: Extirpation of the thymus failed to cause death. It did not produce rickets or any disease of the skeleton. It was impossible to be certain that it caused any alteration in the animal. A great minority of experiments suggested the possibility that removal of the thymus had caused a retarded or diminished growth of the skeleton, and therefore of the animal as a whole; some experiments suggested that removal had provoked changes in the thyroid in the nature of an hyperplasia, hypertrophy of the suprarenal or retarded development of the testes. There is a cogent reason, however, which we now wish to emphasize for believing that the changes in the various parts of the body mentioned were actually due to some other cause than deprivation of thymus function, namely, because they did not occur in combination in the same animals. For example, dogs Nos. 8, 30, 39 and 67 showed retardation in growth, dogs Nos. 7 and 10 showed the underdevelopment of the testes, dogs Nos. 1, 8, 10, 67, 70, 72, 73 and 74 exhibited the most marked variation from the control animals in the thyroids, and dogs Nos. 13, 12, 63 and 70 in the weights of the suprarenals. If any dog in our experiments suffered from the effects of thymectomy, it was dog No. 8. He showed the most marked retardation of growth and the most marked variation in the finer anatomy of the thyroid of all of our thymectomized animals, but no retardation in the development of the testes and little if any hypertrophy of the adrenal.

GENERAL SUMMARY

As the result of our experiments it is possible to draw some general conclusions in regard to the function of the thymus in the dog. Thymus function is absolutely unessential to life. Thymus function is not necessary for the normal process of ossification. Thymus function is not required for the normal growth and development of the hair, teeth and muscles and for the normal intelligence. It is necessary to point out, however, that even if there had not been a single change in any of the organs of internal secretion in the animals of our experiments, it would still have been impossible for us to state that the destruction of thymus function does not produce any changes in them for the reason that our investigations did not cover the period which immedi-

ately followed thymectomy. It may well be that changes were present in the endocrine glands of the thymectomzed dogs of our experiments but had completely vanished by the time the organs were examined, or had been reduced to mere traces (the changes in the thyroids?). Attention has never been directed to the possibility that the period immediately following thymectomy may be the one in which alterations in the endocrine glands are best developed or may be the only one in which they are present. All investigators have awaited the development of symptoms of thymus insufficiency in the living animal.

It now remains for us to attempt to explain the positive findings of other investigators. Though we can do no more than speculate concerning their origin, we hazard the opinion that ultimately the view will receive general acceptance that the changes ascribed by many investigators and perhaps by all to deprivation of thymus function have nothing to do with loss of thymus function but were the result of chance variations or of the effects of confinement or other unfavorable environmental influences or of unsuitable food or disease on animals whose vitality already had been lowered by the shock of a severe operation.

We have previously indicated that many of those who have reported characteristic changes in the living animal after thymectomy or in the skeleton or organs after death seem to have proceeded from the assumption that all deviation from the standards set by the control animals was necessarily the result of deprivation of thymus function, and that the operation of thymectomy was a factor altogether negligible which did not render the thymectomized animal any more susceptible to disease than the normal animal or any less resistant to a variety of injurious influences. We have already pointed out, however, that no two animals are exactly alike in size or form or in the morphology of the internal organs even though they are of the same litter and of selected stock, and hence chance variations are bound to appear, and further, that in the offspring of mongrel stock—such as has been used, we should judge, by all investigators without exception—normal variation has extremely wide limits. We have also indicated that the operation itself is always the cause of temporary retardation in growth or development through the entrance of infection, the existence of which may never be recognized. We have also endeavored to make clear that the animal confined in a cage or cellar, while still in a weakened condition resulting from the operation, or given unsuitable food, or infected with mange or round worms or distemper may be unable to rally and so remains feeble and retarded in growth and development and, perhaps, finally dies while the control animal remains unequally affected or is not affected at all. On reflection, how absurd it seems to suppose that ten animals, whose thoraces were opened

when they were only a few days old and a large structure dissected from the beating heart, the great nerve trunks and vessels of thorax and neck should, once they had survived the immediate danger to life, all grow as rapidly and thrive as well as the animals which had remained untouched, and that the ultimate mortality in the one group should be no whit greater than in the other. Experimental results such as those obtained in the rabbit by Lucien and Parisot, or by Basch in the dog, consisting essentially in temporary retardation of growth, could be most naturally and simply explained as the direct or indirect effects of the operation, or of the operation and confinement. Expermental results like those of Tarulli and Lo Monaco, Cozzolino and Fulci, in which one or two thymectomized animals out of a group failed to grow at the normal rate and developed skeletal changes of one sort or another are certainly susceptible of interpretation on the ground that the one or two animals, whose powers of resistance had been most reduced by the operation, were the ones least able to throw off infection or withstand environmental conditions or disease. There can be no doubt that the positive findings of Matti and Klose, in which a considerable proportion of the thymectomized dogs were affected and finally died are explicable on the same general principles. We do not pretend to assert that none of the positive experimental results, which have been reported by other workers, after thymectomy, could have been due to deprivation of thymus function. *We merely point out that there are other explanations than deprivation of thymus function for the symptoms and pathologic changes which have been reported in thymectomized animals, and that those explanations must be seriously considered in the interpretation of all positive experimental findings, and that for the interpretation of the positive experimental findings reported by some investigators those explanations become absolutely essential.*

In order to obtain a proper perspective in regard to the work which has been done on the subject of thymus extirpation up to the present time, therefore, it is most important to realize that there is nothing so peculiar or remarkable about the kind of positive experimental results reported as to impart to them a specific significance. Disturbances in the growth and development of the skeleton such as retardation in growth, pseudo-rickets or true rickets, are not infrequently encountered in young animals as spontaneous developments and constitute, we might say, the regular form of response of the growing animal accustomed to natural conditions when subjected to the various influences of captivity. That wild animals in zoological gardens are especially prone to develop rickets or ricketslike affections of the skeleton is a fact of too general knowledge to require comment.

Rickets, or, at least, a pathologic condition of the skeleton resembling rickets, has been described by Findlay[187] as a development in normal puppies confined in cages. An outbreak of true rickets of unexplained origin among laboratory rats has been described by Pappenheimer.[23] Rickets in the rat thought to have been caused by an infection has been described by Morpurgo[188] and rickets in the dog, also ascribed to infection, has been described by Koch.[189] Rickets, or a closely related pathologic condition of the bones appeared in both thymectomized and control dogs of the experiments of Renton and Robertson. Rickets, or a very similar condition, developed in one of the sick control animals and in one of the sick thymectomized animals of our experiments. Rickets-like deformities of the extremities appeared in two normal puppies belonging to us that were confined in cages. It is interesting to note also that diminution in the calcium content of the bones, cyst formation, and spontaneous fractures have been reported in animals after hypophysectomy, that lesions in the skeleton resembling those of spontaneous rickets have been reported after parathyroidectomy,[190] and that rachitis has been described after removal of the carotid bodies (Betke[191]). According to Klose[32] diseases of the skeleton in the nature of an osteomalacia have been observed in confined animals following severe operations not involving the organs of internal secretion. Klose writes: "Pawlow observed them (i. e., pathologic conditions of the skeleton in the nature of osteomalacia) after various kinds of operations in the pancreas region and after the production of intestinal fistulae, and Fischler and Looser after operations on the bile ducts.[192]

187. Findlay, L.: The Etiology of Rickets: a Clinical and Experimental Study, Brit. M. J. **2**:13, 1908.

188. Morpurgo, B.: Ueber eine infectiöse Form der Osteomalacie bei weissen Ratten, Beitr. z. path. Anat. u. z. allg. Path. **28**:620, 1900. Di una forma infettiva di osteomalacia nei ratti albini, Atti d. r. Acad. d. Fisiocrit, **12**:59, 1900. Durch Infection hervorgerufene malacische und rachitische Skelettveränderungen an jungen weissen Ratten, Centralbl. f. allg. Path. u path. Anat. **13**:113, 1902. Studio esperimentale sul' osteomalacia e sulla rachitide, Arch. per le sc. med. **31**: 1, 1907. Also transl., Abstr., Arch. de méd. expér et d'anat. path. **19**:119, 1907. L'ostéomalacie et le rachitisme expérimentaux, Arch. de méd. expér. et d'anat .path. **19**:119, 1907. Ueber die infektiöse Osteomalacie und Rachitis der weissen Ratten, Verhandl. d. deutsch. path. Gesellsch. **11**:282, 1908.

189. Koch, J.: Zur Aetiologie der Rachitis. Ueber die Beeinflussung des Knochenwachstums durch bakterielle Infektion, Centralbl. f. Bakteriol. [etc.], 1. Abt. **57**:250, 1913. Ueber experimentelle Rachitis, Berl. klin. Wchnschr. **51**:773, 836, 886, 1914.

190. Frangenheim, P.: Die Krankheiten des Knochensystems im Kindesalter, Stuttg., 1913, F. Enke. 349 p. 8°. N. deutsche Chir., hrsg. von P. von Bruns, Bd. x.

191. Betke: Experimentelle Untersuchungen über die physiologische Bedeutung der Glandula carotica, Beitr. z. klin. Chir. **95**:343, 1915.

192. So sah sie Pawlow nach allen möglichen Operationen in der Pankreasgegend und bei Darmfisteln, Fischler und Looser nach solchen an allen Gallenwegen auftreten.

In putting valuations on the results which have been obtained by means of thymus extirpation, therefore, the greatest importance must be given to the fact that the symptoms and pathologic changes which have been ascribed to deprivations of thymus function are almost without exception the symptoms and pathologic changes which occur in laboratory animals as the result of confinement, improper food, unhygienic conditions, bacterial and parasitic infections and are identical with or closely related to those which have been reported after the removal of at least two organs of internal secretion in addition to the thymus, after excision of the carotid bodies and after a number of different abdominal operations.

In order to illustrate the non-specific character of some of the changes attributed to destruction of the thymus, we need only to turn to the experiments of Betke.[191] Betke excised the carotid bodies from dogs and from kittens. He noted no changes for from four to eight weeks, but, after that *latent period* had elapsed, he discovered that the animals deprived of the carotid bodies became *retarded in their growth* and *increasingly weak, lost flesh* and *passed into a state of cachexia,* and *finally, months after the operation, died.* At the necropsy the animals showed both *macroscopic* and *microscopic evidences of rickets and changes in the spleen similar* to those described by Flesch in his thymectomized rats. If it is true that the carotid bodies are merely aggregations of chromaffin tissues, as is generally thought, and as Betke himself seems to consider probable, it is difficult to believe that they could be necessary to life or have any great importance, especially when it is remembered that life can continue after all of the medullary portions of both suprarenals have been removed.[193] Schmidt[194] found that removal of both carotid bodies in animals produced no effect. Nevertheless, Betke drew from his experiments the conclusions that the carotid bodies are organs of internal secertion, and are essential to life, and further, that they belong to that group of endocrine glands which preside over the growth and development of the skeleton. Thus the results obtained by Betke from removal of the carotid bodies are identical in their main features with the results obtained by Klose and Matti from removal of the thymus, and the conclusions drawn by Betke from his experiments are almost exactly the same as the conclusions drawn by Klose and Matti in regard to thymus functions from their experiments! We believe that the positive results reported by most investigators after thymectomy, and, perhaps, those reported by all investigators, have had some other basis

193. Biedl: Innere Sekretion, 1916.
194. Schmidt: Beitr. z. klin. Chir. **88**:301, 1914.

than deprivation of thymus function and have been falsely and in many instances inexcusably interpreted as due to deprivation of thymus function.

Before concluding our paper we wish to make certain suggestions, drawn from our experiences, in regard to the experimental work in thymus extirpation which may be undertaken in the future. We believe that it is certain that extirpation of the thymus does not produce death or cause rickets to develop in the skeleton. We think, however, that the question of the influence of thymus extirpation on growth and development and of the effects of thymus extirpation on certain of the organs of internal secretion, notably the thyroid, testis and suprarenal, are not settled. For the study of the effects of thymus extirpation on growth and development we suggest that only thoroughbred stock, standardized in point of size, be used, that each thymectomized animal have his own control animal of the same sex and of the same litter and that the animals be not confined nor placed in large groups, but be kept under conditions which are absolutely natural for the species of animal employed. We believe that the problem of the effects of thymectomy on growth and development can never be solved unless the experimental conditions just mentioned are complied with rigidly. For the determination of the effects of thymectomy on the organs of internal secretion we suggest that studies of the organs of internal secretion be made in the first few weeks which follow thymectomy as well as at a later period and that each thymectomized animal have his own control animal of the same sex, and that the conditions under which the animals are placed be normal ones. We urge, further, that the completeness of the thymectomy be judged solely by the experience of the operator at the time of operation and by the results of the most careful examination of the animal at necropsy for thymus rests, and that all experiments in which there is every evidence that the thymus was completely extirpated, be regarded as valid, whether the results happen to meet the preconceived ideas of the investigator or not. We believe that positive conclusions in regard to the effects of thymectomy should be drawn only from evidence which is overwhelming. We cannot refrain from expressing the hope that the habit which has recently sprung up of exorcising into being phantom thymus rests whenever the results of experimental thymus extirpation are negative will cease.

CONCLUSIONS

We conclude as follows:

The thymus gland is not essential to life in the dog.

Extirpation of the thymus produces no detectable alteration in the hair, teeth, contour of the body, muscular development, strength, activity or intelligence of the experimental animal.

Extirpation of the thymus probably does not influence growth or development. The possibility that it may cause retardation in development and delayed closure of the epiphyses, however, cannot be excluded absolutely.

Extirpation of the thymus probably produces no alterations in the organs of internal secretion. It is possible that it produces well marked changes in the organs of internal secretion in the period immediately following thymectomy which was not covered in our experiments.

The writers wish to express their thanks to Dr. Duesberg for his assistance in the examination of the microscopic sections of the testes, and to Dr. Goetsch for his assistance in the examination of the microscopic sections of the thyroid, and to Professor Howland for his advice and assistance on too many occasions and in too many ways to permit mention.

PROTOCOLS OF EXPERIMENTS

EXPERIMENT 1.—Litter 1, composed of dogs 1 and 1, control; mongrels; both females. Born Oct. 21, 1911.

TABLE 1.—EXPERIMENTAL DATA OF LITTER 1

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
1	F	7 wks.	8 mo. 25 days	7 mo. 6 days	Sacrificed (with ether) in healthy condition
1 control	F	.....	8 mo. 25 days	7 mo. 6 days	Sacrificed (with ether) in healthy condition

Dec. 9, 1912: Operation on dog 1, 7 weeks old; still suckling. Intratracheal anesthesia. Thymus apparently removed completely from thorax and neck. Postoperative condition good. Weights of dogs not taken until December 16.

December 10: Dog 1 walks about with difficulty, but feeds greedily.

December 12: Dog 1 stronger but less active than control.

December 16: Superficial portion of wound gapes; wound dressed. Weight: dog 1, 1,220 gm.; control, 1,240 gm.

December 20: Recovery from operation appears to be complete, except that wound has not healed entirely.

Jan. 3, 1913: Weight: dog 1, 1,820 gm.; control, 1,830 gm.

January 18: Weight: dog 1, 2,030 gm.; control, 2,020 gm. Thymectomized dog more shy than the control, but shows no other difference.

Feb. 8, 1913: Weight: dog 1, 3,250 gm.; control, 2,854 gm. Condition of thymectomized animal is normal in every respect.

February 21: Weight: dog 1, 3,850 gm.; control, 3,550 gm. Three sinuses still persist in the thymectomized animal, but its general health is not affected in any way.

March 1, 1913: Weight: dog 1, 4,200 gm.; control, 3,960 gm. No difference is noted in the dogs.

March 12: Weight: dog 1, 4,720 gm.; control, 4,350 gm. Sinuses in dog 1 are as in the previous note; the thymectomized dog is normal in other respects.

May 28, 1913: Weight: dog 1, 7,020 gm.; control, 6,950 gm. Condition of thymectomized dog is normal; the sinuses are closed. Roentgenograms of the extremities of both animals show no abnormalities.

June 11, 1913: Weight: dog 1, 6,800 gm.; control, 7,080 gm. Condition of thymectomized animal is normal. Heretofore the animals were kept in a stable opening into a small yard. On this date they were sent to the farm of the Rockefeller Institute, where they were confined outdoors in a kennel opening into an inclosure measuring about 20 by 15 feet.

July 15, 1913: Weight: dog 1, 7,140 gm.; control, 7,170 gm. On this date both animals were returned from the Rockefeller Institute farm. Thymectomized dog is still entirely normal, showing no differences from the control, except that she is much more shy. Both animals were killed with chloroform and necropsied.

NECROPSY, DOG 1, JULY 16, 1913.—The dog was killed with chloroform. It was a well nourished animal; perfectly formed, showing no deformities or decrease in rigidity of the bones of the extremities. The hair and teeth were normal. The subcutaneous fat was abundant. The pericardium and those parts of the upper lobes of the lungs lying immediately behind the sternum were found adherent to the latter. As adhesions were separated a considerable quantity of fatty tissue was disclosed on the pericardium and about the great vessels. Pieces were dissected away for serial sectioning. On dissecting about the great vessels, we found a mass of tissue above and behind the left subclavian vein, close to its junction with the left innominate vein, which somewhat resembled thymus tissue. It was saved for serial section. All organs seemed to be entirely normal.

Bones.—Femur, gross appearance: A normally formed, strong, hard bone. Sectioning must be done with the saw. On examining the section through the lower end, near the middle it is seen that the cortex is more than 2 mm. thick and that the epiphysis is still open. The zone of proliferative cartilage in the microscopic preparation at hand is not much more than a line in thickness and forms an undulating curve. Microscopic examination, lower end: Columns of cartilage cells are visible at the sides with abundant deposition of calcium in the intercellular substance between them, but in the more central portions the cells have largely vanished, only columns of calcified intercellular substance remaining, with blood vessels and marrow elements lying between them and showing evidences of bone formation. The trabeculae appear to be normal, but the eosin stain is too deep to make the differentiation of the osteoid border possible. Marrow is normal; in the epiphysis it is largely replaced with fat. On examination of section through the upper end complete fusion of the epiphyseal and diaphyseal elements has taken place. The trabeculae are normal. Osteoid margins along the trabeculae are not present. Marrow in the epiphysis is almost wholly replaced by fat; in the diaphysis it is normal.

NECROPSY, DOG 1 CONTROL, JULY 16, 1913.—This was also a well nourished dog. Thymus was large and sent a process behind the left subclavian vein, close to its termination. Weight 7 gm. The organs present a normal gross appearance.

Bones.—Femur, gross appearance: Development in the part of the lower end of the bone from which the microscopic preparation was taken (vertical anteroposterior section through condyle) appears to be a little behind that of the thymectomized animal. Proliferative zone of cartilage bordering on the shaft is a little broader than in the thymectomized animal; the cell columns are deeper and everywhere present. Calcium deposition is in the form of a thin band lying along the diaphyseal border of the cartilage while the rest of the proliferative cartilage still remains calcium free. No osteoid borders are visible.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 1

Spleen.—Malpighian bodies are visible as small blue points, slightly larger in the control than in the thymectomized animal. They present pale staining centers made up chiefly of larger cells than in the peripheral portions, with pale nuclei and pink cytoplasm. Some nuclear fragments are present. Pulp cells are diminished in number. Reticular cells are increased in number; also, the connective tissue elements. Some multinucleated giant cells are seen, but they are not very numerous. Nuclear fragments are present in the pulp; there is some phagocytosis. Little blood is present in the sinuses or veins. The trabeculae are more numerous than is normal. Section of the spleen of the control dog shows essentially the same condition.

Suprarenals.—Architecture of the cortex and medulla in the control and thymectomized dogs is identical. No difference in the morphology of the cells is noted. The respective width of the medullae in stained preparations cannot be compared because the sections in the two dogs were taken in different planes.

Pancreas.—Section from the thymectomized animal is normal; that from the control presents the same appearance, except at one point where there is dense infiltration with polymorphonuclear leukocytes of the connective tissue around one of the ducts and extending into the parenchyma.

Hypophysis.—The gland of the thymectomized dog is extremely vascular, but appears to be normal. The hypophysis of the control was not taken.

Thyroid.—The general arrangement of the thyroid of the control animal has a lacework appearance. The acini are moderately large in the majority of cases and characteristic. Throughout the section the acinar wall shows a very early beginning infolding, giving the appearance of a moderate degree of hyperplasia. The cells have a columnar appearance and are rather rich in protoplasm. The nuclei are rich in chromatin. There is a moderate amount of colloid. There is no increase of connective tissue. There is no striking amount of interstitial tissue, nor are there many newly formed acini. The section from the thyroid of the thymectomized animal presents the same characteristic appearance throughout, but the acini are smaller, on the whole, than in the control. The walls of the acini are more wavy and irregular; there are more small acini present, and there is an abundance of cellular stroma between the acini and in the acinar walls. These interacinar cells are apparently epithelial cells, in large measure; at any rate they have the appearance much like that of true parenchymal cells. The general impression is that there is a further degree of hyperplasia present in this section than in that of the control. A moderate amount of colloid is present. There is an appearance of increased vascularity also. The vessels are readily observed throughout the section. There is no increase in connective tissue.

Ovaries.—No differences were detected in the ovaries.

Thymus.—The thymus of the control shows no atrophy. The cortex and medulla are of equal thickness. Numerous Hassall's corpuscles were noted.

Tissues from dog 1 saved for examination by serial section for thymus rests: No thymus rests were found. Every fourth section was examined with the mechanical stage.

TABLE 2.—BODY WEIGHTS AND WEIGHTS OF ORGANS OF LITTER 1

	Body Weight, Gm.	Spleen, Gm.	Liver, Gm.	Kidneys, Gm.	Suprarenals, Gm.	Thyroids, Gm.	Thymus, Gm.
Dog 1.....	7,140	12.0	244.0	39.5	1.0	1.5	...
Control.....	7,170	15.5	220.0	37.2	1.1	2.5	7.0

EXPERIMENT 2.—Litter 2, composed of dogs 4 and 5 and a control; mongrels (mother a cocker spaniel); all females; born Jan. 16, 1913.

TABLE 3.—EXPERIMENTAL DATA OF LITTER 2

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
4	F	12 days	8 mo. 23 days	8 mo. 11 days	Sacrificed (with ether) in healthy condition
5	F	16 days	4 mo. 10 days	4 mo.	Sacrificed (with ether) in healthy condition
Control	F	.....	8 mo. 23 days	8 mo. 11 days	Sacrificed (with ether) in healthy condition

Jan. 28, 1913: Operation on dog 4, 12 days old. After the usual preparation an incision was made in the median line extending from the base of the neck to the lower part of the sternum; the incision carried through the sternum. On retraction of the divided parts the beating heart appeared in the lower angle of the incision. Internal mammary vessels were ligated. Thymus was removed by blunt dissection; it was found to send a prolongation to the esophagus. Removal was thought to be complete. The operation was conducted under ether, with the aid of intratracheal insufflation, according to the Meltzer method. Weight: thymus, 1.8 gm.

January 29: Condition good. Suckles well. Walks with some difficulty. Respiration accelerated.

January 30: Condition excellent; except for the bandage there is little indication that an operation had been performed.

January 30: Operation was done on dog 5, 14 days old. It was conducted as in the case of dog 4. The thymus was thought to be removed completely. Postoperative condition of dog was good. Weight of thymus, 2.4 gm.

Feb. 3, 1913: Dog 5 has almost entirely recovered from the operation. Weight: dog 4, 725 gm.; dog 5, 635 gm.; control, 601 gm.

February 4: Roentgenograms of the extremities of all three animals were made. No changes were noted.

February 10: Weight: thymectomized dog 4, 910 gm.; thymectomized dog 5, 885 gm.; control, 795 gm.

Dog 4 was not quite so active as dog 5 or the control, but both operative animals seem to be normal. Both suckle vigorously.

February 17: Weight: dog 4, 1,105 gm.; dog 5, 1,156 gm.; control, 1,020 gm. Condition of both thymectomized animals was normal.

February 21: Weight: dog 4, 1,530 gm.; dog 5, 1,555 gm.; control, 1,405 gm. The line of incision has disappeared almost entirely in both thymectomized animals. The behavior of all three is the same. They now are receiving bread and milk in addition to their suckled milk.

March 3, 1913: Weight: dog 4, 1,757 gm.; dog 5, 1,927 gm.; control, 1,705 gm. Except that dog 4 is less active than dog 5 and the control, no changes were noted. Gait of both thymectomized animals was normal. The rigidity of the bones of the extremities had decreased.

March 10: Weight: dog 4, 2,290 gm.; dog 5, 2,375 gm.; control, 2,100 gm. Both thymectomized animals have a normal appearance and behavior.

March 17 and 24, April 1, 8 and 15, 1913: On these dates all three animals were carefully examined and no changes were noted.

April 22: Weight: dog 4, 2,978 gm.; dog 5, 3,170 gm.; control not taken. The dogs were photographed on this date.

May 26, 1913: Weight: dog 4, 6,060 gm.; dog 5, 6,120 gm.; control, 5,920 gm. Both thymectomized animals were normal. Roentgenograms of the extremities were taken. Dog 5 was killed with ether. The necropsy was performed immediately.

June 7, 1913: Weight: dog 4, 6,300 gm.; control, 6,100 gm. Condition of dog 4, normal. Up to this time the animals had been kept in a stable opening into a small yard, but on this date they were sent to the Rockefeller Institute farm.

July 14, 1913: Weight: dog 4, 6,690 gm.; control, 6,363 gm.

Sept. 20, 1913: Weight: dog 4, 6,643 gm.; control, 6,755 gm. Dog 4 appears to be normal in every respect.

Oct. 11, 1913: Weight: dog 4, 6,690 gm.; control, 7,727 gm. Dog 4 was slightly thinner than the control, but was exceedingly active, running and leaping about, and not showing the slightest deformity of the extremities. His hair was glossy. On this date both animals were killed with ether, and the necropsy was performed immediately. Dog 5 was killed 4 months, dog 4 and the control 8 months and 11 days, after operation.

NECROPSY, DOG 5, MAY 26, 1913.—All findings were normal. A small bit of tissue, not larger than a pea, lying just above the left innominate vein, proved to be thymus on examination of serial sections.

Bones.—Ribs and femur: Normal on gross and microscopic examination.

NECROPSY, DOG 4, OCT. 11, 1913.—Body of normal appearing dog. Hair was glossy. The extremities were delicately formed. The rigidity of the humeri was the same as that in the control. The subcutaneous fat was scant. The thyroids and parathyroids were accidentally not saved. The thorax was asymmetrical. On opening the latter no thymus tissue was found anywhere; none was seen on dissecting along the course of the jugular veins. A small mass of fatty tissue which it was thought might contain thymus tissue, a small mass just below the junction of the innominate veins and another at the junction, resembling lymphoid tissue, were saved for serial section. Heart and lungs were normal. The spleen was pink; marked with numerous small yellow spots[195] varying in size from a mere point to 1 or 2 mm. in diameter. On section the cut surface also shows these yellow spots. The malpighian bodies were quite prominent. The kidneys filled with small yellow masses like those found in the spleen; they have the consistency of cheese; otherwise the kidneys are normal. The suprarenals are normal. The liver presents a normal appearance, except for the presence of numerous small yellow masses scattered throughout its substance. The pancreas, intestines, stomach, ovaries, hypophysis and brain are normal.

Bones.—Ribs, gross appearance: Normal contour. No pathologic enlargement of costochondral junction. Can be cut lengthwise without much difficulty. Line of junction of shaft and cartilage approximately straight and clearly defined. Cortex thought to be of normal thickness. Microscopic: Cartilage normal in its differentiation and arrangement. The half dozen or more cells of the columns of cartilage which lie nearest the shaft are large, square or oblong, and have a vacuolated appearance, containing only shrivelled nuclei.[196] Calcium deposition in the cartilage absolutely uniform. Columns of calcified cartilaginous substance of shaft continuous with the calcified intercellular substance of the cartilage are numerous, and with their cross branches form an elaborate lattice work. Bone formation along their sides can be

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195. Dr. Theobald Smith was good enough to examine for us slides from the yellowish areas and from those of dogs 4, control, 8, 8, control, and 70. He reported "It resembles to a certain extent tuberculosis, but more a lesion which I have found in various organs of dogs due to a small undetermined nematode. Such lesions I have found in various organs, including the thyroid, and in some sections the worm was definitely determined."

196. The normal appearance for cartilage cells bordering on shaft in a growing bone.

detected a short distance away from costochondral junctions (about the width of preparatory zone of calcification of the cartilage), but layer of bone along sides of calcified columns does not become well marked until a point farther down in shaft is reached. Invasion of cartilage by marrow capillaries is proceeding in orderly manner. Trabeculae of bone absolutely normal, the larger of them exhibiting in places the normal, thin osteoid border. Marrow normal.

Femur, gross appearance: A normally formed, strong, hard bone. On section of lower end fusion of diaphysis and epiphysis found to be complete. Cortex about 2 mm. thick. Microscopic: Proliferative zone of cartilage has entirely vanished, but the remnants of calcified intercellular substance, persisting in the trabeculae, mark its site. Trabeculae normal, showing no osteoid borders. Marrow in epiphysis has largely disappeared; in diaphyseal part of bone present and normal but contains considerable fat. On section through head and neck of femur fusion of epiphysis and diaphysis has occurred.

NECROPSY, DOG 4, CONTROL, OCT. 11, 1913.—Animal shows some yellow masses dotting kidneys and liver as described in the case of dog 4; not found in the spleen. (These yellow masses were larger and more numerous in thymectomized dog.) Thymus fairly large. Malpighian bodies in spleen slightly larger than those in spleen of dog 4, appearing on cut surface like grains of sand. Otherwise necropsy findings in case of dog 4, control, correspond with those of thymectomized dog 4.

Bones.—Ribs, gross appearance: Junction of cartilage and shaft forms slightly irregular line due to fact that the shaft indents cartilage at two points. Demarcation sharp. On microscopic examination conditions found to be normal, but give impression that growth has practically ceased. The calcium deposition is in the form of a thin band, is very dense and encases the cells of cartilage. Very few trabeculae connect with the cartilage, and those which do so have well developed bone lamellae. The marrow bordering on cartilage shows no signs of invading the latter.

Femur, lower end, gross appearance: Fusion of epiphysis and diaphysis has occurred. Microscopic examination: Very little calcified substance is left in trabeculae to mark site of cartilage. Trabeculae normal. No osteoid.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 2

The malpighian bodies in stained preparation from dog 4, control, are slightly larger than those from thymectomized animal. In both specimens malpighian bodies show hyperplasia but are not especially large. Spleens of both animals appear normal and similar to each other.

Suprarenals.—Impossible to make any comparisons in regard to width of medullae of suprarenals in stained preparations because plane of sections is different. Morphology of cells of both cortex and medulla and architecture of the glands are identical in both, and normal.

Pancreatic Glands.—Normal.

Hypophysis.—Normal.

Thyroids and *parathyroids, ovaries* and *thymus* of control not saved.

Tissues from dog 4 saved for examination in serial section for thymus rests: No thymus rests found. Every fourth section examined with mechanical stage. Thymus rest as seen in serial section of tissues from dog 5 normal. Proportions of cortex and medulla normal. No mitoses seen. No signs of involution; none of regeneration.

Weights of organs of dog 4 not taken. Thyroids of the control together weighed 3.5 gm., the largest weight for the thyroids in the entire series of animals. Thymus of control weighed 7 gm.

EXPERIMENT 3.—Litter 3, composed of dogs 7 and 7, control, mongrels, with strong predominance of Great Dane, both males, born March 31, 1913.

TABLE 4.—EXPERIMENTAL DATA OF LITTER 3

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
7	M	26 days	16 mo. 9 days	15 mo. 13 days	Sacrificed (with ether) when not fully recovered from attack of distemper
7 control	M	.....	16 mo. 9 days	15 mo. 13 days	Sacrificed (with ether) when practically well after distemper

April 25, 1913: Operation on dog 7, 26 days old. Intratracheal anesthesia. Thymus thought to be extirpated completely. Slight hemorrhage. Thymus weighs 5.1 gm. Postoperative condition good. Weight: dog 7, 1,470 gm.; control, 1,645 gm. (see Fig. 8).

May 20, 1913: Wound has healed and animal presents an appearance differing in no respect from control. Weight: dog 7, 3,770 gm.; control, 3,600 gm.

May 28: Condition dog 7, normal. Roentgenograms of extremities of both animals show normal appearance. Weight: dog 7, 4,900 gm.; control, 4,840 gm.

June 7, 1913: Thymectomized animal seems normal. Sent to Rockefeller Institute farm. Weight: dog 7, 6,400 gm.; control, 6,570 gm.

July 13, 1913: Weight: dog 7, 8,181 gm.; control, 8,408 gm.

Sept. 20, 1913: Dog 7 shows no abnormalities; is quite as active as control. Weight: dog 7, 12,727 gm.; control, 12,954 gm.

Oct. 24, 1913: Condition normal. Weight: dog 7, 16,236 gm.; control, 16,236 gm.

Dec. 14, 1913: Both animals photographed. Weight: dog 7, 19,307 gm.; control, 18,408 gm.

Jan. 18, 1914: No abnormalities of any sort can be found in thymectomized animal. Weight: dog 7, 20,227 gm.; control, 19,317 gm.

Feb. 8, 1914: Weight: dog 7, 22,158 gm.; control, 19,545 gm.

March 20, 1914: The thymectomized animal is a splendid example of a normal dog. Weight: dog 7, 22,954 gm.; control, 22,954 gm.

July 4, 1914: Since the last note both animals have had distemper, dog 7 more severely than the control. Both animals are slightly affected by the mange. Because dog 7 continually jumped over a wire netting 6 feet high which separated him from some of the smaller animals, it was necessary to chain him in his pen, so that in the past three months he has had much less exercise than the control dog. Weight: dog 7, 21,600 gm.; control, 21,800 gm.

Aug. 7, 1914: Both animals killed with ether and necropsied immediately. It was not possible to obtain the weights of either animal (broken scales); dog 7 seemed to be lighter than the control, though it was thought there was not more than 2 kg. difference between their weights. Period of observation, 15 months, 13 days.

NECROPSY, DOG 7, AUG. 7, 1914.—Well nourished animal, showing small spot of mange on forehead and some mucopurulent discharge from eyes and nose. Hair somewhat drier than hair of control and can be pulled out more easily. No deformity or diminution in rigidity of extremities. Abundant subcutaneous fat. Silk ligatures still present at site of operation. Thyroids appear normal. The left measures 12 by 8 by 4 mm.; the right 10 by 5 by 3 mm. Parathyroids thought to be normal in size. On opening thorax a single adhesion was found joining upper part of left lung to sternum. Large amount of fat in region normally occupied by thymus. Fat, on section, contains no tissue resembling thymus; portions, however, saved for serial section. Considerable fat along jugular veins in neck, but no tissue resembling thymus. Heart normal. Lungs normal. Abdominal cavity also contains considerable fat in

omentum and mesentery, and about kidneys. Spleen pink, normal consistency; malpighian bodies not enlarged. Kidneys normal except for presence of numerous small, yellow masses, most of them of pinhead size.[197] Some of these yellow masses have soft centers. The suprarenals also appear normal; right suprarenal measures 2.5 by 1.3 cm.; left suprarenal 2.5 by 1.5 cm. Cortex of both suprarenals about 3 mm. wide; medulla in most places about as wide as cortex; medulla dark brown; cortex yellow. Liver appears normal. Pancreas also normal. Intestines and stomach present normal appearance.

Bones.—Ribs, gross: Normally formed. Contour of costochondral junctions normal. Consistency not hard; little force required to cut. Cut section shows line of demarcation of cartilage and shaft to be sharp. In the two microscopic preparations at hand the line of junction is not straight. In one it is arched, and in the other it is notched at several points so that it is irregular. The cortex is judged to be of normal thickness, but is a little less than that of the rib of the control. The marrow is pale. Microscopic: The cartilage presents a normal appearance as to staining and general arrangement. Calcium deposition between the columns of cartilage cells normal. The cartilage, or at least the calcified intercellular substance of the cartilage, is continuous with the trabeculae of the shaft at relatively few points. Invasion of the cartilage by the marrow is, however, apparently still continuing. Trabeculae thin, not very numerous, and thought to be undergoing absorption at several points, but without agency of osteoclasts. A few of them have traces of osteoid border. In the rib showing the irregular line of junction of cartilage and shaft endochondral ossification is apparently proceeding everywhere in an orderly and uniform manner. The rib does not show the slightest sign of rickets.

Femur, Gross: A large perfectly formed bone, hard and strong. On section of the lower end with the saw it is seen that fusion of the epiphysis with diaphysis has already taken place. Cortex normal thickness. Microscopic: The preparation taken through the lateral portion of one of the condyles fails to show anything abnormal. Trabeculae absolutely normal. Marrow replaced largely by fat.

NECROPSY, DOG 7, CONTROL, AUG. 7, 1914.—Body slightly larger than body of dog 7, and fat is more abundant. Thyroids and parathyroids about the same size as those of dog 7. Thymus very large (not weighed). Spleen light red; malpighian bodies larger than malpighian bodies in spleen of dog 7. Suprarenals about the same size as suprarenals of dog 7, but medulla wider than that of dog 7 in places; medulla of gland three times as wide as cortex; it is dark reddish brown; cortex light yellow. Kidney studded with small yellow masses, such as described in kidneys of dog 7.[198] In other respects necropsy shows nothing noteworthy.

Bones.—Ribs and Femur: Gross and Microscopic: Normal. Not differing in any respect from conditions found in bones of thymectomized dog, except that osteoid border of trabeculae of rib is considerably broader in the control and quite generally present, and the trabeculae are more numerous and show no signs of rarefying process. Femurs of the two dogs are equal in size; cortices apparently equal in thickness.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 3

Spleen.—The malpighian bodies appear about equal size in the microscopic preparations of both dogs (slightly larger in control), and show in both signs of hyperplasia. No well marked differences can be detected.

Suprarenals.—Comparing the numerous microscopic preparations taken from different cross-sections in both dogs, the maximum width of the medulla is

197. See footnote 195.
198. See footnote 195.

approximately the same. Considerable variation in width of medulla found in series from each animal; in some sections no medulla is visible on microscopic examination, whereas in others it is more than 2 mm. wide. Architecture of the cortex and morphology of the cortical cells essentially same in both; no difference in arrangement or appearance of cells of medullae.

Pancreatic Glands.—Normal. No differences can be detected.

Hypophysis of dog 7 has normal appearance. That of control not sectioned.

Parathyroids of both dogs show small areas of sclerosis; otherwise normal.

Thyroids.—The thyroid of dog 7 shows an abundance of colloid. The majority of the acini are quite large with very much thinned out acinar epithelium. The lining cells are flat, the nuclei are also flattened out; evidently there is increased tension in the colloid acini. Here and there are areas of more cellular thyroid tissue in which mere drops of colloid are seen. There is certainly no indication of hyperplasia in this section, if anything, quite the contrary. The section from thyroid of dog 7, control, shows a typical simple colloid picture, with a striking regularity of the acini, which latter contain a considerable amount of colloid with atrophy of acinar walls, the epithelium of which is flat. There is an absence of vascularity and a striking absence of anything suggesting hyperplasia in any part of the section. A very small amount of interstitial tissue is present.

Testes.—Testes of dog 7 show large number of giant cells which are apparently spermatids fused together. No spermatozoa seen anywhere; none present in epididymis. Testes of dog 7, control, on the other hand, are fully developed, normal organs. Spermatozoa present in large numbers in both testes and epididymis.

Thymus.—Thymus of dog 7, control, not examined.

Tissue saved for examination in serial section for thymus rests not studied. The animal was so large and such masses of fat were present in the neck that microscopic examination for thymus rests of a relatively extremely small portion of the fat seemed useless. All tissues saved for serial section appeared in the gross to be fat.

TABLE 5.—Body Weights and Weights of Organs of Litter 3.

	Body Weight, Gm.	Spleen, Gm.	Kidneys, Gm.	Liver, Gm.	Supra-renals, Gm.	Thy-roids, Gm.	Testes with Epididymes, Gm.	Pan-creas, Gm.	Brain, Gm.
Dog 7.....	21,600*	28.0	74(?)	682.0	1.5	2.5	15.7	54.0	87.0
Control.....	21,772*	50.0	95	600.0	1.8	2.6	28.0	50.0	85.0

* The weights of dogs 7 and 7, control, were not taken at the necropsy. The weights given are the last weights taken, one month before the animals' deaths. During the last month dog 7 undoubtedly lost weight.

Experiment 4.—Litter 4, composed of dogs 8 and 8, control, mongrel puppies, both males and constituting the entire litter; born April 7, 1913.

TABLE 6.—Experimental Data of Litter 4.

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
8	M	19 days	10 mo. 1 day	9 mo. 12 days	Killed by another animal while in perfect health
8 control	M	.....	10 mo. 6 days	9 mo. 17 days	Sacrificed (with ether) in healthy condition

April 26, 1913: Operation on dog 8, aged 19 days. Thymus thought to be completely extirpated; moderate hemorrhage; immediate postoperative condition of animal, however, good. Weight: dog 8, 1,140 gm.; control, 1,180 gm. (Fig. 10).

May 20, 1913: Thymectomized animal in excellent condition. Wound entirely healed. For a time dog 8 walked with forelegs spread wider apart than control, but after wound had healed this peculiarity in gait ceased. No diminished rigidity of long bones of leg. Weight: thymectomized animal, 1,930 gm.; control, 2,470 gm.

May 28, 1913: Roentgenograms of extremities of both animals taken. Weight: thymectomized animal, 2,220 gm.; control, 2,860 gm.

June 11, 1913: Condition of both animals the same, 8, control, remaining slightly the heavier. Absolutely no abnormalities in hair, teeth, gait or extremities of dog 8. Behavior the same. Both sent to Rockefeller Institute farm. Weight: dog 8, 3,030 gm.; control, 3,880 gm.

July 13, 1913: Still no differences discernible in the two animals, except that dog 8, control, appears to be growing faster than his operative mate; the latter, however, is in every respect as active and appears to be as healthy. Weight: dog 8, 3,175 gm.; control, 4,648 gm.

Sept. 20, 1913: Dog 8 so small that it was necessary to remove him from the inclosure in which he was formerly kept with his control and some larger animals, for the reason that he was constantly attacked by the others. He is the most active animal of the entire group at the farm, and shows no abnormality anywhere. Limbs perfectly formed but very slender; skeleton of this animal much more delicately made than that of control. Weight: dog 8, 3,175 gm.; control, 4,648 gm.

Sept. 20, 1913: Dog 8 as in previous note. Weight: dog 8, 4,308 gm.; control, 7,257 gm.

Nov. 24, 1913: Still dog 8 shows no pathologic condition of any kind unless his slender build be regarded as such. Exceedingly agile, stealing other animals' food, etc. Weight: dog 8, 3,864 gm.; control, 7,927 gm.

Dec. 14, 1913: Dog 8 still shows no abnormality except in size and build. Both dogs photographed. Weight: dog 8, 5,215 gm.; control, 9,184 gm.

Jan. 18, 1914: The thymectomized animal looks extremely well, and continues to show no abnormalities. On account of great difference in his development and that of control he has been examined with greatest care without bringing to light any abnormal condition anywhere other than the slender proportions already alluded to. Weight: thymectomized animal, 4,989 gm.; control, 9,297 gm.

Feb. 8, 1914: On this date dog 8 was bitten to death by one of the larger dogs. Weight: dog 8, 5,328 gm.; control, 10,886 gm.

NECROPSY, DOG 8, FEB. 10, 1914.—Dog killed by another dog February 8. The injuries produced are as follows: Great extravasation of blood in subcutaneous tissues over upper part of thorax, extensive lacerations without hemorrhage of tissue of the neck. Rupture of the right sternocleidomastoid muscle. Dislocation of the right thyroid. Fractures of seventh and eighth ribs with extensive laceration of thoracic wall. Right hemothorax. Laceration of lower lobe of right lung. Rupture of diaphragm. Laceration of right lobe of liver, of right suprarenal and head of pancreas. Body kept frozen.

Body that of a fairly nourished animal. Frame lightly made, giving appearance of slenderness but not of disease. Extremities delicate, normal contour. Bones of extremities have normal rigidity. Skin intact; hair normal. Teeth normal. Considerable fat in subcutaneous tissue.

On opening thorax right lung was adherent in its upper part to posterior surface of manubrium. Right pleural cavity free from adhesions. Left pleural cavity normal. Considerable amount of fat covers pericardium and found in neck. On account of extravasation of blood which extends upward into subcutaneous tissues of neck impossible to make perfect dissection of latter. Great vessels, however, can be followed upward and region of thyroids examined. No tissue resembling thymus can be found along great vessels of neck, about thyroids, on surface of the pericardium, or about great vessels of thorax. All tissues from these regions which could possibly be suspected of containing

thymus removed for serial section. Thyroids appear large, but have a normal color and appearance on section. Heart normal. Left lung normal. Right lung normal except for extensive laceration of lower lobe. Mesenteric lymph nodes not enlarged. Testes normal. Brain normal. Hypophysis appears normal. Spleen presents normal external appearance. On section color red; malpighian bodies well defined, not abnormally prominent; pulp has normal appearance, consistency normal. Both large and small intestines show no abnormality; lymphatic elements not hyperplastic. Left suprarenal fairly large (section made lengthwise through entire organ); cortex 1 mm. in width, medulla 2½ mm., cortex bright yellow, medulla brown. Pancreas normal. Liver normal except for laceration of right lobe already mentioned.

Bones.—Ribs, gross appearance: Slender, normal contour at costochondral junctions. Cut with some resistance. Line of union of cartilage and shaft sharply outlined and approximately straight. Cortex thought to have usual thickness. Microscopic: Cartilage normal. Columns rather long; arrangement everywhere parallel. The nine or ten cells nearest shaft large, square, rounded or oblong, apparently empty, except for a shrunken, degenerated nucleus. Calcium deposition between columns of cartilage cells absolutely regular and extending farther into cartilage than is seen in most of other dogs of equal age of his series. Columns of calcified substance of shaft adjacent to cartilage have a strictly parallel arrangement and close to the cartilage are separated from each other by only the width of cartilage cell. A short distance from the cartilage many end abruptly or become connected with adjacent columns so that they are there less numerous. Apposition of bone can be detected along them close to the cartilage, but the lamellae of bone formed on them do not become thick until a short distance removed from the cartilage border. Signs of an orderly invasion of the cells of the cartilage columns by marrow vessels passing toward them between the columns of calcified substance can be seen in numerous places. Many trabeculae, the larger ones in particular, show on one side, or here and there, an osteoid border of a width not exceeding the normal for the growing dog. The architecture and finer histology of the trabeculae normal. Periosteal bone formation normal. Marrow normal (Fig. 24).

Microscopic preparations from two additional ribs show pictures identical with above.

Femur, gross appearance: Normal contour. For section sawing is required. On examination of several different sections through lower end of bone a thin band of epiphyseal cartilage hardly more than a line can be seen in each. The cortex is about 2 mm. thick.

Microscopic: The thin band of epiphyseal cartilage marking off the termination of shaft is about 1 mm. wide in stained preparation, and intact throughout. In one preparation taken through the condyle it has more or less the shape of the letter M; in another, taken more nearly between the condyles, it forms an obtuse angle pointing epiphysealward. It is composed of parallel columns of cells which extend almost to the epiphyseal bone. The morphology of the cells of columns normal. Calcification of cartilage normal, calcium deposition being limited to the intercellular substance between columns. Cartilage sends many stalactite-like prolongations of calcified intercellular substance into the shaft, most of which show large bone formation along their sides. Marrow here and there can be seen to be invading cartilage. Trabeculae of bone in both epiphysis and diaphysis normal. They do not show any osteoid borders, except here and there thin rims along some of the broader trabeculae near the cartilage. Fat has replaced the marrow almost entirely in epiphysis, and very largely in diaphysis (Figs. 25 and 26).

Two microscopic preparations taken at different vertical anteroposterior planes through the upper end of tibia show similar pictures. Proliferative zone of cartilage still persists along the end of shaft and has a normal arrange-

ment. Calcium deposition in it uniform. Osteoid margins are more numerous among the trabeculae of two preparations from the tibia than in those of femur.

NECROPSY, DOG 8, CONTROL, FEB. 13, 1914.—Dog killed with ether. Necropsy performed immediately. Weight, 10,900 gm. Well nourished, perfectly formed dog. Primary incision shows abundance of subcutaneous fat, more than thymectomized dog 8 possessed.

Thymus presents normal appearance. Thyroids and parathyroids normal. Thyroids somewhat larger than thyroids of thymectomized animal; show no abnormalities. Parathyroids normal.

Spleen normal in size; surface blue; cut surface dark red; malpighian bodies appear as small white areas about the same in size and number per unit of surface as appear in spleen of thymectomized animal. Suprarenals present normal appearance; right considerably larger than left. Right suprarenal cut in cross-section presents yellow cortex of about 1 mm. in width and a medulla averaging 1 mm. Considerable variation in width of medulla at different points. Left suprarenal cut longitudinally like suprarenals of thymectomized dog 8. Cortex measures about 1 mm. in width and the medulla from 2 to 3 mm. On gross examination it shows no essential difference from the left suprarenal of thymectomized dog 8. Hypophysis appears normal and shows no difference from that of thymectomized dog.

Bones.—Ribs and lower end of femur, gross and microscopic. Normal but larger than those of dog 8. Preparations from ribs show fully as prominent osteoid margins along the trabeculae as the preparations from ribs of thymectomized animal. There is this difference between the femora of two animals in addition to disparity in size, that in the case of control, diaphysis and epiphysis have fused completely, but in the thymectomized, as already pointed out, the two are still separate.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 4

Spleen.—Malpighian bodies in stained preparations slightly more prominent in control than thymectomized animal, but not very large in either. Spleens of both animals appear normal, showing no essential differences.

Suprarenals.—Architecture of suprarenals of two animals appear to be normal and the same. Medullae cannot be compared with reference to width in microscopic preparations because latter are taken from longitudinal sections of gland cut in different though parallel planes. Structure of medullary cells in dog 8 appears normal, but stroma elements are mingled with them, due to the fact that the section was made through medulla close to corticomedullary junction. In medulla of control cut through middle, cells form solid mass.

Hypophysis.—In both animals hypophysis appears normal.

Parathyroids.—Preparations from control are normal. Those from thymectomized dog show most marked degree of sclerosis. Thick and thin strands and branching processes of fibrous tissue divide gland into innumerable parts. In some of these parts arrangement and appearance of parathyroid cells is approximately normal, but in most places the parathyroid cells appear to be crowded together, and are heaped up into masses of different sizes and shapes. Some of these are round, crescentic or strandlike in outline, and vary, as seen of course in cross-section, from aggregations of a dozen or more cells to large numbers which could not possibly be counted. The cells in these densely packed areas have a different appearance from the parathyroid cells in parts of gland less affected. Nuclei smaller, more irregular in shape; some of them pyknotic, stain dark blue. In strands of fibrous tissue blood vessels with thickened walls are seen. In some of these intimal proliferation has taken place.

Thyroids.—The thyroids of the thymectomized animal presents a rather irregular picture, i. e., there are areas in which the acini are moderately large and

fairly regular, and alternating between these areas and others in which the thyroid acini have almost lost their regular contour and arrangement, and in which there is an apparent increase in the parenchymal cells without a formation of definite acini. In these latter areas there is practically no colloid present. In the areas of more normal looking thyroid, acini are quite regular, epithelium is cuboidal type, nuclei large, rich in chromatin, and acini contain considerable amount of colloid. In general, impression is obtained that there are areas in this gland which truly simulate focal hyperplasia in an otherwise fairly normal looking gland. The section of thyroid from control presents a very normal picture. The acini are very regular and provided with colloid. Epithelium cuboidal, nuclei supplied with chromatin; stain well. Small amounts of interstitial thyroid substance present, but no evidence of hyperplasia. Picture that of a simple, normal, colloid gland.

Testes.—Preservation poor. No late stages of spermatogenesis present in the testes of either dog. Process of spermatogenesis in dog 8 appears to be in same stage of development as in dog 8, control.

Thymus.—Thymus of dog 8, control, is a normal gland showing practically no signs of involution. Some fat between the lobules. The cortex and medulla are about equal in thickness. Numerous Hassall's corpuscles.

The serial section of the tissues collected saved for examination for thymus rests: No thymus tissue present. Every fourth section examined, using mechanical stage.

TABLE 7.—BODY WEIGHTS AND WEIGHTS OF ORGANS OF LITTER 4.

	Body Weight, Gm.	Heart, Gm.	Spleen, Gm.	Kidneys, Gm.	Liver, Gm.	Suprarenals, Gm.	Thyroids, Gm.	Testes, Gm.	Thymus, Gm.
Dog 8.....	5,340	39.5	8.25	26.5	170.0	0.5* (left)	0.5	9.5	...
Control.....	10,900	85.0	12.2	50.0	395.0	1.35 (both)	0.8	10.7	3.5†
Ratio: Oper.:Control	1 : 2.04	1 : 2.15	1 : 1.48	1 : 1.8	1 : 2.32	1 : 1.35‡	1 : 1.6	1 : 1.1	

* Right adrenal lacerated and incomplete.
† Weight only approximate. Before fat was separated weight was 11.5 gm.
‡ Weight of left adrenal of dog 8 divided into half the weight of both adrenals of the control animal.

EXPERIMENT 5.—Litter 5, composed of dogs 10, 13 and common control, all three males, constituting group A; and of 12 and 12, control, both females, constituting group B, born May 8, 1913, of mongrel stock, in which bulldog predominates.

TABLE 8.—EXPERIMENTAL DATA OF LITTER 5.

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
				Group A	
10	M	9 days	7 mo. 12 days	7 mo. 3 days	Died as result of severe ascaris infection, having also mange and abscess in thorax
13*	M	15 days	12 mo. 7 days	11 mo. 22 days	Sacrificed (with ether) while still sick with distemper. Mange
10C†	M	.....	11 mo. 21 days	11 mo. 6 days	Sacrificed (with ether) when dying from distemper pneumonia
				Group B	
12	F	11 days	12 mo. 6 days	11 mo. 25 days	Sacrificed (with ether) in healthy condition
12C	F	.....	12 mo. 6 days	11 mo. 25 days	Sacrificed (with ether) in healthy condition

* Originally dog 13 had a separate control from this same litter, 13C, but the latter died of ascaris infection when 5 months old. He has been dropped from the experiment.
† Dog 10C serves as control for dog 13.

Group A.—May 17, 1913: Operation on dog 10, 9 days old. Thymus thought to be completely removed. Weight: 1.8 gm. Postoperative condition good. Animal not weighed until May 23.

May 23: Operation on dog 13, 115 days old. Removal of thymus thought to be complete. Slight hemorrhage. Postoperative condition good. Weight: dog 10, 980 gm.; dog 13, 1,260 gm.; control, 1,240 gm. (Fig. 9).

May 26: Both thymectomized animals in excellent condition. Weight: dog 10, 1,220 gm.; dog 13, 1,410 gm.; control, 1,180 gm.

June 17, 1913: Condition of both thymectomized animals continues excellent. Wound of dog 13 entirely healed, but sinus has appeared in anterior part of line of incision of dog 10; sinus about 2 mm. in diameter and discharges yellow pus. Probe inserted through sinus enters small cavity in superficial tissues by compression of which pus can be obtained. In spite of presence of discharging sinus dog 10 very playful. Weight: dog 10, 2,400 gm.; dog 13, 2,590 gm.; control, 2,470 gm.

June 30: Both thymectomized animals behave in normal manner. Sinus of dog 10 continues to discharge. Neither of the two thyymectomized animals show any peculiarity in gait or behavior as compared with control. Legs of thymectomized animal straight, showing no enlargement at knee. On seeking to bend between hands long bones of forelegs of the three animals it is found that no differences in rigidity exist. Weight: dog 10, 3,200 gm.; dog 13, 3,530 gm.; control, 3,130 gm.

July 13, 1913: Still no differences between thymectomized and control dogs Sinus of dog 10 continues to discharge. Weight: dog 10, 3,285 gm.; dog 13, 3,630 gm.; control, 3,175 gm.

Sept. 20, 1913: Both thymectomized animals active and intelligent, showing no abnormalities of any kind. Dog 10, sinus still discharges. Weight: dog 10, 6,000 gm.; dog 13, 5,624 gm.; control, 5,966 gm.

Nov. 24, 1913: Since last note dog 10 has lost flesh, so that he now appears thinner than dog 13 or the control. Mange has begun to develop. The sinus persists. Dog is both intelligent and active, running when called, and competing with the others in obtaining food. He looks thin but not sick. Dog 13 in all respects normal. Weight: dog 10, 4,317 gm.; dog 13, 5,227 gm.; control, 6,136 gm.

Dec. 14, 1913: Dog 10 has become still thinner, so that he now has emaciated appearance. Mange has become very extensive in spite of treatment and given rise to purulent conjunctivitis. Both corneae show opacities. Animal now lies in kennel and will not come out when called. When lifted up and set down on his feet he at once lies down; takes his food poorly, seems stupid. Ribs outlined through skin and legs appear delicately fashioned; no enlargements at knees. Long bones of extremities do not bend easily. Sinus continues to discharge. Dog 13 and control continue to be normal except for mange. Weight: dog 13, 7,257 gm.; control, 7,250 gm.

December 20: Dog 10 died.

Jan. 18, 1914: Dog 13 and control both normal except for the mange, and almost indistinguishable from each other. Weight: dog 13, 7,813 gm.; control, 7,954 gm.

March 20, 1914: Weight: dog 13, 9,772 gm.; control, 8,863 gm.

March 29: Dog 13 has become severely affected by the mange, so that whole body is involved. Otherwise healthy, vigorous animal.

April 29, 1914: Control killed with ether. Two days previously he was taken acutely sick with distemper which appeared in epidemic form among the animals, and was killed when in a moribund condition. Weight: control, 7,393 gm. Dog 13 has begun to snuffle, apparently beginning to develop distemper.

May 15, 1915: Dog 13 killed. Since last note he developed distemper and had not yet recovered though he had improved. He looked sick and snuffled continually, but was active, running freely about, barking and eating normally.

Mange showed no tendency to diminish under treatment. Dog never showed any abnormality of skeleton, perversion of appetite or diminution of intelligence up to time of death.

Dog 10 observed 7 months and 3 days; dog 13, 11 months and 22 days after operation.

NECROPSY, DOG 10, MARCH 29, 1914.—Small male dog, showing extreme emaciation; purulent discharge from eyes. Scar of operation not visible. Small sinus covered by scab marks line of incision. On incision through soft parts sinus found not to extend to bone or into chest, as had been thought. No pus but granulation tissue present. Thyroids normal in size and pale. Jugular veins and carotid arteries dissected to junctions with large vessels of thorax. Small piece of yellowish tissue picked from right jugular at its lower end to be examined in serial section for thymus. Comparatively few adhesions bind lungs to anterior chest wall; upper lobes, however, quite adherent to each other in the midline, but can be separated. Small piece of yellowish tissue removed from right mammary artery not larger than head of pin, and a small piece about size of pea removed from right subclavian; both saved for serial section. Besides these no tissue in any way resembling thymus found. Entire right middle lobe of lung shows marked pneumonia. Patches of pneumonia in other lobes. Heart dilated. Spleen very small and pale. Liver dark brown and small. Adrenals appear normal in size and color. Testes also appear normal. On opening stomach and intestines former contains mass of hair and a few round worms, with some undigested food; latter is literally packed with round worms, in places greatly distending the lumen. No undigested food and no feces in the intestines. Examination of brain and hypophysis not made.

Bones.—Ribs, gross appearance: Slender, but perfectly formed. They can be split with a sharp knife rather easily. On section the line of junction of shaft and cartilage is perfectly distinct, forming almost but not quite a straight line. Cortex well defined but perhaps a little thin. Microscopic: Cartilage normal. Columns of cartilage cells parallel but not very long. Calcium deposition in part contiguous with shaft normal, forming narrow continuous band stretching from one side to the other and completely enveloping the cells; stains an intense blue. At two points edge of the calcified cartilage rimmed for short distance with bone. Marrow adjacent to cartilage normal and shows no tendency to invade latter. Trabeculae, continuous with cartilage, few in number and show well marked bone formation at points close to cartilage. Trabeculae appear normal. No osteoid visible. Marrow contains considerable number of large round giant cells; osteoclasts only occasionally seen (Fig. 28).

Femur: Normal contour, hard, so that sawing is required. Apparently strong. A thin zone of epiphyseal cartilage persists on border of shaft; in an anteroposterior section, taken through the condyle, it has the shape of an M, and in another section, taken between the condyles, forms almost a straight line. Cortex a little more than 1 mm. thick. Microscopic: Cartilage normal; columns, which are parallel, occupy two thirds of breadth of zone. Calcium deposition normal; appears as intensely blue fringe arranged along the diaphyseal border of cartilage. At all points it completely fills the ground substances between the columns of cells, and in many places is found in the ground substance between the separate cells in some columns. Marrow very largely disappeared. Marrow cells fringe some of the trabeculae and lie along the capillaries, but are so reduced in number that the supporting framework is clearly outlined. Trabeculae extremely thin and diminished in number. In numerous places they are undergoing destruction, which seems to be taking place without the agency of osteoclasts. No osteoid border is visible. The arrangement of the trabecule is normal (Fig. 36).

NECROPSY, DOG 13, MAY 15, 1914.—Dog killed with ether. Necropsy performed twenty-four hours after death. Poorly nourished dog, covered with

lesions of mange. Frothy discharge about nostrils and mucopurulent discharge from eyes. On primary incision little subcutaneous fat. Both lungs adherent to posterior surface of sternum. On account of small amount of fat it was thought thymus rests, if present, could be found easily, but no tissue resembling thymus seen anywhere. Heart normal. Lungs normal. Thyroid glands large, soft, pink, normal on section. Parathyroids identified, thought to be normal in size. Suprarenals normal; medulla about 2 mm. wide at widest point; cortex slightly narrower than medulla. Kidneys normal. Pancreas normal. Liver normal. Testes normal. Mesenteric and bronchial lymph nodes hyperplastic. Stomach and intestines normal. A mass of round worms found in upper part of small intestine and in stomach. Brain normal. Hypophysis normal.

Bones.—Ribs, gross appearance: Entirely normal so far as external examination is concerned; can be split with a knife, but only with considerable pressure. Line of costochondral junction clear cut, in one specimen at right angles to shaft and straight, in another arched. Cortex normal width. Microscopic: Cartilage normal. Calcification of cartilage normal. Columns of cartilage cells relatively long. Calcium deposition extends relatively far into cartilarge. Columns of calcified cartilaginous substance of shaft adjacent to cartilage numerous, fairly thickly placed, and having an orderly arrangement. Bone formation along these columns is not very marked close to cartilage, but is normal. Here and there a trabecula showing a thin rim of osteoid at one or more points can be found.

Femur, gross appearance, lower end: Normal, except that in shaft close to the thin band of epiphyseal cartilage, which still persists, is an abscess containing in addition to pus cells numerous fragments of necrotic bone and some masses of necrotic cartilage. No osteoid along trabeculae visible. Columnar arrangement of cartilage orderly and calcium deposition in it normal. Upper end: Head of femur and great trochanter not yet fused with shaft. In diaphysis close to epiphyseal junction, and also in epiphysis close to the zone of proliferating cartilage, there are a number of areas in which the marrow shows focal necrosis, but the trabeculae of bone about them remain normal. No osteoid. Cartilage normal and calcium deposition in cartilage normal.

NECROPSY, DOG 10, CONTROL, APRIL 30, 1914.—Dog killed with ether when in a moribund condition. Necropsy twenty-four hours after death. Animal moderately well nourished. Some subcutaneous fat. On opening thorax and dissecting neck, thymus much atrophied, of reddish brown color, resting on left innominate vein and sending thin prolongation along left jugular vein for about 2 cm. into neck; very edematous. Right lung shows pneumonic consolidation of right middle lobe. Spleen large, light red in color; on section malpighian bodies scarcely distinguishable. All other organs normal except liver, which is thought to show focal necrosis.

Bones.—Gross and microscopically: Only lower end of femur examined, and it shows an absolutely normal condition. A zone of cartilage is present, not quite so wide as that in the lower femoral epiphysis of 10, control, and of about the same width as that of dog 13. No osteoid. Marrow very largely replaced by fat.

Group B.—May 19, 1913: Operation on dog 12, 11 days old; removal of thymus thought to be complete. Weight of thymus not recorded. On right side short blunt process of thymus found extending up into neck along great vessels. Postoperative condition good. Weight: dog 12, 960 gm. Dog 12, control, not weighed until May 22 (Fig. 9).

May 22: Condition of thymectomized animal excellent. Seems to have almost entirely recovered from operation; suckles with avidity. Weight: dog 12, 1,020 gm.; control, 1,120 gm.

May 26: Condition of dog 12 excellent. Recovery from operation complete. Dressings removed; wound clean; stitches withdrawn. Roentgenograms of extremities of both animals show no changes. Weight: dog 12, 1,390 gm.; control, 1,440 gm.

June 16, 1913: Dog 12 entirely normal, not differing from control in any respect. No disturbance in gait and no diminution in rigidity of long bones of extremities. Weight: dog 12, 2,690 gm.; control, 2,730 gm.

June 30: Still no differences in appearance or behavior of two animals. Both growing rapidly. Weight: dog 12, 3,350 gm.; control, 3,180 gm.

Sept. 20, 1913: Dog 12 continues normal. Weight: dog 12, 5,510 gm.; control, 6,419 gm.

Nov. 24, 1913: Dog 12 still normal, alert and active; still not showing any differences from control. Weight: dog 12, 7,499 gm.; control, 7,045 gm.

Dec. 14, 1913: Roentgenograms of extremities of both animals show normal conditions. Weight: dog 12, 9,070 gm.; control, 8,160 gm.

Jan. 18, 1914: Absolutely no difference except in marking and coloring. Both splendid examples of normal vigorous dogs. Weight: dog 12, 8,939 gm.; control, 8,237 gm.

Feb. 8, 1914: Both animals in splendid condition. Weight: dog 12, 11,107 gm.; control, 10,147 gm.

March 20, 1914: Dog 12 remains normal. Weight: dog 12, 11,913 gm.; control, 11,793 gm.

May 14, 1914: Both animals killed with ether in perfect health.

NECROPSY, DOG 12, MAY 14, 1914.—Body of normally formed, healthy, well nourished dog. Limbs perfectly developed. Subcutaneous fat about 1.5 cm. thick. On opening thorax lungs adherent to posterior surface of sternum by firm adhesions broken with difficulty. Most careful search for thymus rests in both thorax and neck reveals none. A great deal of fat lying at points of normal situation of thymus, and especially along jugular vein, removed for serial section in possibility that thymus rests might be present in its substance. Thyroid large, dark in color. Near upper pole of left thyroid, on posterior lateral aspect, is parathyroid surrounded by whitish zone of fat. Gland of right side the same size as left. At upper pole a body similar to that on left side, obviously parathyroid. On section thyroids soft; cut surface pink. Heart normal. Lungs normal. Spleen red, firm; on section malpighian bodies prominent; some almost 1 mm. in diameter, tending to stand out from cut surface. Mesenteric glands not enlarged. Mucous membrane dark pink; intestinal wall throughout of uniform thickness; mucosa of large intestine shows a few much injected areas; two round worms found; lymphatic elements distinguished with difficulty. Kidneys normal.

Suprarenals.—Left: Cortex about 2 mm. thick, medulla about same thickness in widest part; latter dark brown; cortex yellow. Right suprarenal corresponds to left, having the same general relationship between cortex and medulla. Liver normal. Ovaries normal. Brain and hypophysis of normal appearance.

Bones.—Ribs, gross appearance: Apparently normal. Costochondral junctions show no enlargements. Section from ribs not taken.

Femur, gross appearance: Normal contour hard, so that it must be sawed, and strong. On section cortex between 2 and 3 mm. thick. Epiphysis and diaphysis fused at both ends of bone. Microscopic (lower end): Epiphyseal and diaphyseal portions of bone can be distinguished from each other by the greater marrow content of the latter, which makes it appear dark, and by the different arrangement of the trabeculae. Articular cartilage normal in thickness. Trabeculae are normal. Some of them show here and there the slightest trace of osteoid border. Marrow in epiphyseal part of bone almost entirely replaced by fat; marrow in diaphyseal portion contains considerable fat. Cortex is normal. In another microscopic preparation of lower end of femur, taken from a point between the condyles, a band of cartilage separating nucleus

of ossification of epiphysis from diaphysis still present but extremely narrow. Its columns of cartilage cells have parallel arrangement and calcium deposition normal. Section taken through upper end of femur shows normal condition. Fusion of head and femur with neck complete. Trabeculae normal. No osteoid. Cortex of bone thick.

NECROPSY, DOG 12, CONTROL, MAY 14, 1914.—A well nourished, normal appearing dog. Less subcutaneous fat found than in dog 12. Thymus composed of tough reddish tissue and of fairly large size (weighed about 5 gm.), easily identified, quite distinct from fat. The spleen more tender and friable than spleen of dog 12, and the malpighian bodies not so numerous. Suprarenals correspond almost exactly to those of dog 12.

Bones.—Femur: Femur alone examined. Section through upper end showed no abnormalities. Fusion of upper and lower epiphyses has taken place.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF BOTH GROUPS OF DOGS OF LITTER 5

The medullae of the suprarenals are not very thick in any of the five animals of this litter, and are about equal at their widest points, as shown by a comparison of the numerous microscopic preparations taken from various cross sections. Architecture of cortex and proportions of the three zones to each other, and morphology of cells, essentially the same in all. Structure of medulla in dog 10 is normal; in dog 10, control, and dog 13 degenerative changes in the medulla have taken place. The structure of the medulla in dogs 12 and 12, control, appears to be normal and identical.

Pancreas.—Pancreatic glands of dogs 10, control, and 12 and 12, control, normal. Gland of dog 13 shows extensive infiltration of the interstitial tissue with leukocytes, most marked in the neighborhood of ducts, and in dog 10 this condition is so marked as to constitute a diffuse suppurative pancreatitis.

Spleen.—The microscopic preparations from the spleens of dogs 12 and 12, control, are similar. The malpighian bodies slightly larger in dog 12, and appear on gross inspection of stained preparations from that animal as small blue points, whereas in spleen of dog 12, control, they are barely visible. Both spleens contain much blood. Spleen of dog 10 atrophic as might have been expected from the wasted condition of the animal, but at the same time shows on microscopic examination some hyperplasia of the malpighian bodies, and large numbers of polymorphonuclear leukocytes scattered through the pulp. The spleens of dogs 13 and 10, control, have exceeedingly small malpighian bodies, which are not visible except on examination with the microscope Spleens of both dogs show similar pictures and appear normal.

Parathyroids.—Those from dog 12 appear normal. In the parathyroids of dog 12, control, many of the blood vessels seem to be accompanied by relatively abundant connective tissue investments, so that the vascular arrangement is brought into great prominence, and the parenchymatous elements separated from each other appear in the form of strands, rings, and irregularly shaped masses. The parenchymatous elements appear normal. In dog 10, control, similar condition to that just described exists; no one of parathyroids of these three dogs shows colloid.

Thyroids.—Section from the thyroid of dog 12 presents perfectly normal, regular, colloid appearance; there is the normal amount of interstitial tissue present; no areas of hyperplasia seen anywhere. Thyroid of dog 12, control, gives general impression of slight increase in amount of colloid. Acini fairly large in many instances. Epithelium thin. At one edge of section there is an area of gland in which the acini are smaller, and there is more cellular increase. In this portion small acini are recognized. No hyperplasia seen. In the section from the thyroid of dog 13 a great deal of desquamation of acinar cells into the lumen of vesicle. Many of these cells are many times their normal size, with an abundant amount of granular protoplasm, and a

larger than normal, centrally placed, dark-staining nucleus. In certain of the areas acinar wall is broken up; in still other areas the structure is fairly regular, the acini rather large with thin epithelium and a good deal of colloid. In areas of parenchymal desquamation the acini are small and contain very little or no colloid. In parts of the section there seem to have been extensive rupture of acini with escape of colloid into the interstitial tissue, giving an edematous appearance. In dog 10 section shows rather dense appearing tissue in which acini on the whole are small. Very few moderate sized acini. The appearance is that of a rather abundant parenchyma with very little colloid. There is no true infolding anywhere to be seen. There are many small and newly formed acini; moderate amount of interstitial tissue. The epithelium everywhere is of cuboidal type. No increase in connective tissue; no increased vascularity.

Ovaries of dogs 12 and 12, control, show no difference.

Testes of dogs 10, 10, control, and 13: Those of dog 10 are still in embryonic stage of development, whereas testes of dogs 10, control, and 13 are the fully developed adult type of gland showing all stages of spermatogenesis.

Thymus, Dog 12, Control.—Microscopic: Thymus of 12, control, shows relatively slight degree of involution. Lobules separated from each other by considerable fat and connective tissue. Medulla and cortex clearly defined and of about equal thickness. Cortex stains deep blue; medulla much lighter colored. In medulla blood vessels appear to be more numerous than in normal gland; filled with blood and do not appear to have thickened walls. Numbers of Hassall's corpuscles exceedingly few, only one large one and two small ones being found in examination of a number of sections. Thymus of dog 10, control, shows extreme atrophy. Lobules widely separated from each other by edematous, fat containing connective tissue. Cortex almost entirely disappeared, being represented in some of lobules by an extremely thin border densely packed with cells, which take a deeper blue stain than rest of lobule. Lobule filled with blood vessels distended with blood, but do not appear to have particularly thickened walls. No Hassall's corpuscles can be seen in any of a large number of sections examined.

Serial sections of tissues for thymus rests from dogs 10, 12 and 13 failed to show the presence of any. Sections were 15 microns thick. Every fourth section examined.

TABLE 9.—Body Weights and Weights of Organs of Litter 5

	Body Weight, Gm.	Spleen, Gm.	Liver, Gm.	Kidneys, Gm.	Suprarenals, Gm.	Thyroids, Gm.	Testes, Gm.	Ovaries, Gm.
Dog 10.....	*	4.5	155.0	28.5	0.75	0.5	2.5	....
Dog 13†.....	8,844	16.6	525.0	60.0	2.3	1.0	11.0	....
10 control†.....	7,398	15.5	510.0	51.0	1.1	1.0	10.5	....
Dog 12†.....	13,550	33.5	635.0	68.0	2.1	1.4	....	0.75
12 control†.....	12,700	26.0	780.0	63.0	1.3	1.3	....	0.8

* Body weight at necropsy not recorded, but undoubtedly under last recorded weight of 4,300 gm. The weights of the adrenals and thyroids are relatively high, those of testes and spleen low. The spleen was greatly atrophied.

† Dog 10 killed when moribund from distemper, and dog 13 sick with distemper when killed; they undoubtedly had lost weight and were in a diseased condition. Dogs 12 and 12, control, were healthy when killed.

Experiment 6.—Litter 6, composed of two dogs, 30, a female, and 30, control, a male, born Oct. 3, 1913, of small mongrel stock, in which black and tan predominate.

Since these two animals were 6 weeks old, and therefore beyond the arbitrary age limit set by Klose for effective thymectomy, it was decided to make removal of the thymus incomplete with a view to subsequent study of the thymus rest.

TABLE 10.—EXPERIMENTAL DATA OF LITTER 6

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
30	F	6 wks.	12 mo. 5 days	10 mo. 21 days	Not perfectly clear, but thought due to diarrhea associated with ascaris infection
30C	M	.....	14 mo. 15 days	13 mo. 1 day	Killed (with ether) in healthy condition

Nov. 16, 1913: Operation on dog 30, 6 weeks old. Thymus completely removed, except a fragment about 3 mm. in diameter. Gland was dissected out of thorax from below upward. During procedure small thin process of thymus tissue, continuous with main mass, could be seen passing above and behind left innominate vein. The end of this process was snipped off and allowed to remain in situ while remainder of gland was removed. Weight: thymus, 6.8 gm.; dog 30, 1,827 gm.; control, 1,340 gm.

November 19: Postoperative condition excellent.

Dec. 16, 1913: Condition of dog 30 normal. Wound entirely healed. No difference between thymectomized and control animals can be deceted. Weight: dog 30, 3,380 gm.; control, 3,340 gm.

Jan. 17, 1914: Dog 30 not so well nourished as control, and thought to have round worm infection. Both animals have developed mange. Weather is extremely cold. Dogs are confined out of doors in a kennel. Dog 30 shows no skeletal changes. Weight: dog 30, 3,471 gm.; control, 3,636 gm.

Feb. 8, 1914: Dog 30 in fair condition, though thin and somewhat mangy. Absolutely no change in skeleton. Weight: dog 30, 4,989 gm.; control, 5,000 gm.

March 20, 1914: Dog 30 as in previous note. Control now heavier, more active and powerful animal. Weight: dog 30, 6,122 gm.; control, 6,818 gm. Although dogs were repeatedly visited, no written observations were made as to weights or condition between this date and the date of death because they seemed normal.

Oct. 7, 1914: Dog 30 died. She sickened the latter part of September and lost considerable weight before death. At necropsy only pathologic condition found was bloody mucus in small intestine without ulceration. She had been treated several times for round worm infection. Dog 30, control, sacrificed Dec. 16, 1914; seemed to be normal except for mange.

NECROPSY, DOG 30, OCT. 7, 1914.—Body of small, very thin dog, showing no external evidence of disease. On primary incision subcutaneous fat exceedingly scant. On opening thorax, tissues, which it is thought may be thymus, found lying on left innominate vein, measuring 1 cm. by 3 mm. by 2 mm.; pink and somewhat edematous. No other tissue resembling thymus seen. Lungs normal. Heart normal. Spleen pink; on section malpighian bodies poorly developed; pulp light red. Suprarenals thought to be normal in size and appearance; medulla and cortex of equal breadth; the former brown, the latter bright yellow. Kidneys normal. Ovaries normal. Pancreas normal. Liver yellowish brown, soft, normal in appearance. Thyroids apparently normal. Parathyroids identified. Hypophysis of normal size. Stomach and intestines normal. Lower part of small intestine contains some reddish brown fluid which resembles changed blood. Mixed in it is considerable sand.

Bones.—Ribs, gross appearance: Slender. Costochondral junctions do not protrude. Bone cuts with little resistance. Cartilage and shaft meet to form a straight line, well defined, at right angles to shaft. Cortex of rib appears thin. Microscopic: Cartilage normal in its structure and staining reactions. It is an interesting fact that on the inner half of rib in the specimen being described, the columnar arrangement of the cartilage is very poorly developed, while in the outer half the cartilage columns are fairly deep and contain large numbers of cells. Growth is apparently proceeding more actively in the outer

half than in the inner half, but probably not very rapidly even there for the deposition of the calcium is such as is seen in a rib in which growth has almost ceased. At one point the edge of the cartilage bordering on the shaft is rimmed with bone. Evidences of very vascular marrow adjacent to cartilage and invasion of latter, and of new trabeculae formation in shaft, wanting. Calcium deposition is, as already indicated, in form of band extending across rib at cartilage border, but with break in continuity at one point. At that one point the cartilage has an homogeneous appearance, containing no cells but presents no other peculiarities. It is not being invaded by the marrow. Trabeculae of shaft normal but probably a little thin. No osteoid. Marrow normal. Section of other ribs shows an absolutely normal picture without the variation recorded (Fig. 37).

Femur, lower end, gross appearance: Normally formed bone which can be cut with a sharp knife but seems fairly hard. Epiphysis fused with diaphysis. Cortex is about 1 mm. thick. On examination of stained specimen it becomes apparent that cartilage separating nucleus of ossification of epiphysis from shaft has not entirely disappeared, and can be seen with the naked eye as an M-shaped line across the bone. It is very faint and is definitely interrupted at several points, but at others stands out clearly. Articular cartilage has normal thickness for adult bone. Microscopic: With magnification it can be seen that cartilage has in reality almost entirely disappeared from the epiphysodiaphyseal junction. That it is visible to the naked eye is due chiefly to remnants of line of calcified cartilage which still persist in middle parts of trabeculae. Trabeculae do not contain osteoid anywhere. The marrow in epiphysis is almost entirely replaced by fat and largely so in that portion of diaphysis bordering on epiphysis.

NECROPSY, DOG 30, CONTROL, DEC. 16, 1914.—Well nourished dog, showing on primary incision abundance of subcutaneous and visceral fat. Thymus large, reaching caudally to auriculoventricular groove and cranially to base of neck. Other organs have normal appearance. Medulla and cortex of adrenals of about equal thickness. Weight: thymus, 3 gm.

Bones.—Ribs and femur, upper end, gross appearance: Normal. Rib shows here and there along trabeculae relatively broad osteoid borders. Calcium depositions in cartilage absolutely normal.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 6

Spleen of dog 30 shows extreme degree of atrophy. Trabeculae lie close together. Pulp very largely disappeared and malpighian bodies not found even with microscope.

Suprarenals.—Comparison of numerous microscopic preparations of cross sections of suprarenals of both animals shows medulla of control slightly wider at widest point than that of thymectomized dog. Cortices in both animals show no differences and appear normal. Medulla of control normal; that of thymectomized dog shows early signs of postmortem degeneration, but, where sufficiently preserved, appears normal.

Hypophysis of dog 30 normal. Hypophysis of control not sectioned.

Parathyroids.—Sections from two animals indistinguishable and appear normal.

Thyroids.—Thyroid of dog 30, control, presents nothing noteworthy. A good deal of colloid present. Considerable diversity in the size of the alveoli, some appearing large and others extremely small. Small alveoli and large also tend to be grouped. No parts of section present solid appearance, however. Epithelium of acinar walls either flat or low cuboidal. No infolding. Some desquamation present. Thyroid of dog 30 presents not very different appearance from that just described. Difference in size of alveoli even greater than

in thyroid of dog 30, control, some very large alveoli being present, but, on the whole, they are smaller. The large alveoli are filled with colloid, but the smaller alveoli are comparatively free from it. No parts of gland present under microscope a solid appearance. The alveolar walls are lined with flat or cuboidal epithelium. In some places the epithelium is swollen and has desquamated. No infolding.

Through an accident the tissues saved for examination for thymus rests were not cut serially. A single section taken from the mass of tissue, which was thought on gross examination to be thymus, was found to contain tissue which somewhat resembled atrophied thymus. No Hassall's bodies were present in it. Cells composing it were large and resembled more or less the reticular cells of the thymus. One of the reasons for thinking the tissue in question might be thymus was that its arrangement appeared to be lobular.

TABLE 11.—WEIGHTS OF ORGANS OF LITTER 6*

	Body Weight, Gm.	Heart, Gm.	Spleen, Gm.	Liver, Gm.	Kidneys, Gm.	Pancreas, Gm.	Suprarenals, Gm.	Thyroids, Gm.	Thymus, Gm.
Dog 30.....	†	32.0	3.0	101.5	15.0	11.0	0.8	0.3	...
Control.....	6,576	61.2	12.4	349.0	45.0	22.0	1.2	0.8	8.0

* Table shows relatively large weight of suprarenal of dog 30, and small weight of spleen, as compared with corresponding organs of control dog.
† Weight at necropsy omitted. It was considerably less than weight of control.

EXPERIMENT 7.—Litter 7, composed of three mongrel puppies, all females, born Oct. 23, 1913, black and tan predominating.

Since this litter was 6 weeks old, the extirpations of the thymus in both dogs 37 and 38 were made intentionally incomplete, with the idea of investigating the question of hyperplasia of the rest.

TABLE 12.—EXPERIMENTAL DATA OF LITTER 7

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
37	F	6 wks.	13 mo. 15 days	12 mo.	Sacrificed (with ether) in a poorly nourished condition and suffering from mange
38	F	6 wks.	2 mo. 24 days	1 mo. 10 days	Died in an emaciated and mangy condition. Abscess in thoracic wall
37C	F	.....	14 mo.	12 mo. 15 days	Sacrificed (with ether) in healthy condition

Dec. 4, 1913: Operation on dogs 37 and 38, both 6 weeks old. In each animal thymus dissected out of thorax from below, and a minute rest left in situ behind and above left innominate vein. In dog 38 amount of tissue left supposed to be thymus so minute that at end of operation doubt was felt whether any thymus tissue had been left. Thymus rest which was left in dog 37 not more than 2 mm. in diameter. Weight: dog 37, 1,700 gm.; dog 38, 1,880 gm.; control, 1,820 gm.

December 16: Both thymectomized animals show skin infections in the neighborhood of operative wound; these latter have healed. Both animals entirely recovered from operation; run and play about with same freedom as control. Weight: dog 37, 1,740 gm.; dog 38, 1,900 gm.; control, 2,140 gm.

Jan. 16, 1914: Since last note the three dogs sent to farm and placed in small kennel out-of-doors. Weather unexpectedly became extremely cold, and animals too young and too recently recovered from operation not to be affected. They remained in kennel practically entire time, venturing out only to feed. Dog 37 and control in poor condition, extremely thin and weak; they

have developed mange. Dog 38 died January 14. He was least vigorous of the three animals and had become severely affected by mange, and developed an abscess in the region of the upper part of sternum. Weight: dog 37, 2,095 gm.; control, 2,150 gm.; dog 38 (January 14), 1,640 gm.

From Jan. 16, 1914, until December, 1914, no written notes were made, though animals were seen at frequent intervals. Dog 37 gradually outstripped control in both size and weight, and became a normal appearing and acting animal, developing no changes in skeleton or elsewhere. Dog 37 was taken ill a few days before he died, Dec. 5, 1914. Mange latterly extremely severe. Control animal sacrificed Dec. 17, 1914. She was smaller than dog 37; she was also affected with mange, though not to the same extent as dog 37, and in a better state of nutrition.

NECROPSY, DOG 38, JAN. 17, 1914.—Body much emaciated; skin excoriated from mange. Wound perfectly healed. Deformity of sternum at junction of fourth costal cartilage in form of knuckle projecting externally. Subcutaneous fat almost entirely absent. As knife passes over knuckle discharge of brownish pus from small abscess in thoracic wall, having no connection with thoracic cavity.

On removal of sternum and costal cartilages yellowish red layer of tissues having something of appearance of thymus seen resting on pericardium below innominate vein; saved in toto for histologic examination. Both pleural cavities entirely free with exception that apex of right lung is slightly adherent to posterior surface of sternum. Behind great veins of thorax are much enlarged lymph nodes. No thymus tissue about veins. Veins and all adjacent tissue, in fact, all loose tissues of upper thorax and lower neck with great vessels themselves removed for serial section. Lungs congested, but show no pneumonia; some atelectasis present. Heart normal. Spleen normal. Kidneys normal. Suprarenals normal; cortex about 1 mm. in width and medulla in most parts represented by line. Liver dark red, normal. Thyroids normal. Ovaries normal.

Bones.—Ribs: Very slender. Can be cut with sharp knife, but offer considerable resistance. Gross appearance: Costochondral junctions have normal contour. On section cartilage and shaft join to form clear cut straight line. Cortex has about normal thickness. Microscopic: Cartilage normal in arrangement and staining reaction. Columns of cells bordering on shaft long, in contrast to condition seen in older animals of this litter, 37 and control. Calcium deposition in cartilage regular and evenly distributed. Trabeculae continuous with cartilage are fairly numerous, and have parallel arrangement. New bone formation normal, well marked, close to cartilage. Trabeculae normal. No osteoid borders visible except along some of broader trabeculae, where in places thin rims of osteoid can be detected. Marrow normal.

Femur, lower end, gross appearance: Femur normally formed, hard, so that a saw is required to cut it. Cortex about 1 mm. thick, sharply marked off from marrow cavity. On section epiphyseal cartilage separating nucleus of ossification of epiphysis from diaphysis is an extremely thin line scarcely 1 mm. thick. Nucleus of ossification occupies almost entire epiphysis but cartilage from 2 to 3 mm. thick lies adjacent to it on one side. Cortex normally thick. Microscopic: Band of epiphyseal cartilage bordering on shaft has columnar arrangement which extends through almost its entire width to epiphyseal side. Calcium deposition in it normal and continuous from one side to other. Trabeculae continuous with cartilage; few in number but normal and parallel. Bone formation well advanced at points close to cartilage. Trabeculae normal. No osteoid. Marrow normal in epiphysis, containing considerable fat.

NECROPSY, DOG 37, DEC. 5, 1914.—Animal poorly nourished, showing extensive loss of hair on forelegs and under parts of body (mange). Skin rough and thickened. No external abnormalities of form. Limbs have normal con-

tour; no enlargements in regions of joints. Small amounts of subcutaneous fat. No signs of any inflammatory lesion at site of operative wound, but thorax in median line forms sharp angle because divided parts of sternum have united at acute angle. Lungs adherent to sternum. Pleural cavities normal, except for pleural adhesions already alluded to. No tissue found resembling thymus; if latter is present it must be in form of minute rests. All light colored tissue in thorax on posterior surface of sternum, as well as in space between and about great veins, along jugular veins in neck and about thyroids, removed for serial section for thymus rests. Heart normal. Lungs normal. Thyroid glands apparently normal. Spleen light red; malpighian bodies very inconspicuous both on surface and cut section. On opening stomach and intestine large numbers of round worms found. Mucous membranes of intestine and stomach show no abnormalities, but in large intestine mixed with fecal matter is blood. Pancreas normal. Kidneys normal. Ovaries normal. Suprarenals rather flat; one measures 19.6 by 10.7 by 6 mm.; medulla at its widest point 1.3 mm., cortex 1 mm. Medulla of other suparenal measures 2.5 mm., cortex 1 mm. Thickness of medulla varies with location of section; where adrenal is round medulla is thick, where broad and thin medulla represented by scarcely more than a line; medulla light brown, cortex yellow. Liver normal. Hypophysis normal. Brain normal.

Bones.—Ribs, gross appearance: Rather slender. Contour costochondral junctions normal; cut with difficulty. Line of junction of cartilage and shaft straight and at right angles to shaft. Cortex of shaft normal thickness. Microscopic: Cartilage normal. Proliferative zone narrow. Calcium deposition heavy, forming band stretching from one side of rib to other. All portions of cartilage bordering on shaft site of calcium deposition, save the interior of cells themselves; latter look like rows of eyes in deep blue background of calcified cartilage. As seen under magnification cartilage bordering on shaft notched at various points so that it appears to present a serrated edge. This edge is in numerous places rimmed with bone. At only two points in the section is cartilage continuous with trabeculae of shaft, and at those two points with old trabeculae of well formed bone, and not with columns of calcified cartilaginous substance. Marrow close to the cartilage normal. Obviously, growth has ceased. Trabeculae themselves normal. No osteoid. Cortex normal.

Femur, lower end: Femur hard and strong, not bending easily and cutting only with saw. Gross: Shape normal. On section lower end, cartilage has disappeared from diaphyso-epiphyseal junction, fusion having occurred, but what was the epiphysis easily distinguished by the different arrangement of trabeculae. Cortex about 1.5 mm. thick, clearly demarcated. Microscopic: Cartilage of epiphysis entirely disappeared except for articular cartilage, which is apparently normal. Trabeculae normal. No osteoid borders. Cortex normal. Marrow of shaft contains much less fat than marrow of what was formerly epiphysis.

NECROPSY, DOGS 37 AND 38, CONTROL,[199] DEC. 17, 1914.—Small, well nourished, healthy looking dog. Thymus represented by thin mass of tissue in anterior mediastinum, atrophied and weighing only 1 gm. Suprarenals measure 19.4 by 9 by 3.8 mm., and 19 by 9.3 by 5.5 mm., respectively. Width of medulla varies much at different parts of gland; where latter is broad and flat it is narrow, appearing as brown line; where it has a more rounded shape, reaches a width of 2.2 mm. No malpighian bodies can be seen on cut surface of spleen. All other organs appear normal.

Bones.—Ribs, gross appearance: These are slender and present no enlargements at costochondral junctions. On section they can be cut with knife, but

199. Although it was the original plan that this dog should be the control of dog 38 also, she cannot be regarded as a control on account of the great difference in age.

are very hard. Junction of cartilage and shaft definite, and in most of ribs forms approximately straight line, but in one rib a line notched at two points. Cortex has normal thickness. Microscopic: Section shows nothing abnormal. Cartilage stains normally and has usual arrangement of cells. Calcium deposition normal. Trabeculae not lined at any point with osteoid.

Femur, gross and microscopic: Lower end and upper end both normal. Fusion of epiphysis with diaphysis has occurred. No trace of osteoid except thin rim visible in places along few of trabeculae. Bone marrow largely replaced by fat.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 7

Spleen.—Malpighian bodies of dogs 37 and control small, but slightly larger in control than in dog 37. Spleens of both animals somewhat atrophic.

Suprarenals.—Medullae of adrenals of dogs 37 and control seem in stained sections about equal in width. Extreme variations present in both sets of specimens. Cortices show no essential differences in architecture or in morphology. Medullae of both show essentially no differences on microscopic examination.

Parathyroids, hypophysis and ovaries of dog 37 appear normal.

Thyroid of dog 37 presents nothing abnormal; in fact, epithelium is cuboidal; acini more uniform; moderately enlarged; moderate amount of colloid present; moderate amount of interstitial tissue. No evidence of hyperplasia. Thyroid of control shows simple colloid picture; acini on the whole fairly large with thinned out epithelium. In certain areas rather marked degree of desquamation of parenchymal epithelium into lumena of acini. No suggestion of hyperplasia. Appearance except for desquamation fairly regular.

Thymus considerably atrophied. Lobules much shrunken, separated by fat and connective tissue. Cortex and medulla can be distinguished, but cortex much condensed. Hassall's corpuscles present.

The serial sections of the tissues collected saved for examination for thymus rests. Every fourth section examined, using mechanical stage. No tissue resembling thymus could be found in the very large numbers of serial sections made from dogs 37 and 38. Every fourth section examined with particular care.

TABLE 13.—BODY WEIGHTS AND WEIGHTS OF ORGANS OF LITTER 7

	Body Weight, Gm.	Heart, Gm.	Spleen, Gm.	Kidneys, Gm.	Liver, Gm.	Suprarenals, Gm.	Thyroids, Gm.	Thymus, Gm.
Dog 37.....	6,130	65.0	6.5	45.3	265	1.1	0.41	...
Control.....	4,308	39.5	9.7	37.0	241	0.97	0.29	1.0

EXPERIMENT 8.—Litter 8, composed of four male dogs, 39, 39 C., 40, 40 C., of medium sized mongrel stock; born Nov. 27, 1913. Mother the same as that of litter 4.

TABLE 14.—EXPERIMENTAL DATA OF LITTER 8

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
39	M	19 days	14 mo. 8 days	13 mo. 19 days	Sacrificed (with ether). Sick for two weeks preceding death with exacerbation of symptoms of distemper
39C	M	.....	14 mo. 15 days	13 mo. 26 days	Sacrificed (with ether) in health
40	M	20 days	14 mo. 15 days	13 mo. 25 days	Sacrificed (with ether) in health
40C	M	.....	7 mo. 13 days	6 mo. 23 days	Died from distemper

Dec. 16, 1913: Operation on dog 39, 19 days old. Thymus thought entirely removed. Operation especially satisfactory. Weight: thymus, 4 gm. Postoperative condition good. Weight: dog 39, 1,140 gm.; control, 950 gm.

December 17: Operation on dog 40, 20 days old. Thymus removed with utmost care. Weight: 3.8 gm. Postoperative condition excellent.

December 18: Weight: dog 40, 1,060 gm.; control, 1,320 gm.

Jan. 4, 1914: Dogs 39 and 40 entirely recovered from operation. Dog 39 fallen behind 39, control, in weight, and dog 40, which weighed less than 40, control, at beginning, now still further behind. Weight: dogs 39, 1,740 gm.; control, 1,975 gm.; dog 40, 1,377 gm.; control, 2,370 gm.

January 13: Since operation all four puppies have been confined in cage 6 by 5 by 1 feet. Roentgenograms of extremities of four animals taken at this time make it appear that femora, tibiae, and also bones of forelegs of dogs 39 and 40 slightly thicker than corresponding long bones of controls. Examination of extremities reveals no differences, however; humeri of thymectomized dogs do not bend easily; no enlargements about joints; no disturbances in gait. All four animals very active; dog 40, the smallest, is only one unable to climb out of cage. Weight: dog 39, 2,450 gm.; control, 2,710 gm.; dog 40, 2,210 gm.; control, 3,230 gm.

Feb. 1, 1914: Puppies still confined in cage, which has become too low for them. No differences found anywhere except that dog 40 continues decidedly smaller than his control. All four dogs sent to country. Weight: dog 39, 3,170 gm.; control, 3,270 gm.; dog 40, 3,200 gm.; control, 4,200 gm.

March 24, 1914: Roentgenograms of extremities again taken, showing that no differences between thymectomized and control animals now exist. Weight: dog 39, 3,939 gm.; control, 4,989 gm.; dog 40, 4,308 gm.; control, 5,896 gm.

July 4, 1916: Dog 39 thought to be developing distemper; known to be present in other animals at farm; eyes and nose both discharging. Dog 40, control, almost 2 kg. heavier than control; the latter, dog 40, however, seems to be healthy and normal in all respects. Since last note dog 39 gained more rapidly than his control in weight. Weight: dog 39, 6,122 gm.; control, 6,576 gm.; dog 40, 7,936 gm.; control, 8,503 gm.

July 10: Dog 40, control, finest specimen in litter, suddenly taken with distemper pneumonia, dying after short illness. Dogs 40, 39 and 39, control, also show signs of distemper, snuffling, and have a mucopurulent conjunctivitis.

Jan. 23, 1915: Dogs 39, 39, control, and 40, brought in from farm. Since last note both thymectomized animals have lost weight, and control has just maintained his. No one of three dogs has been well since attack of distemper. Dog 39 most severely affected at time, has never recovered. All have continued to snuffle, but have been active, running about inclosure. Neither of two thymectomized dogs has behaved in any way differently from control, or has developed deformities of skeleton.

Feb. 5, 1915: Dogs 39 and 39, control, sacrificed with ether. Dog 39 sick after his arrival in laboratory, snuffling more than formerly and losing weight. His weight taken on recorded date considerably less than weight of two weeks ago. Weight: dog 39, 4,308 gm.; control, 6,689 gm.

February 12: Dog 40 sacrificed with ether. Weight, 6,122 gm.

NECROPSY, DOG 39, FEB. 5, 1915.—Body of poorly nourished dog. Hair normal. Contour of limbs, head and trunk normal. On primary incision no fat, but silk stitches marking original line of incision in both soft parts and bone. No inflammatory process. Contour thorax normal. On opening latter, lungs found adherent to posterior surface of sternum. On pericardium and along great vessels in thorax and neck no thymus tissue found. None discovered in region of thyroid glands. The scant fatty tissue in places normally occupied by thymus collected for serial section examination for thymus rests. Heart normal. Lungs show no signs of inflammation. Spleen light red; malpighian bodies seen with great difficulty; cut surface dry. Suprarenals

normal; cortex in both bright yellow, medulla light brown; medulla of left adrenal at lower pole about 2 mm. wide, but elsewhere is narrow; narrow in right adrenal. Kidneys normal. Pancreas normal. Liver normal. Stomach and intestines normal. Few round worms found in upper parts small intestine. No hyperplasia of lymphatic elements of intestine. Mesenteric lymph nodes hyperplastic. Testes normal but small. Thyroids normal, measuring about 2 cm. in length. Parathyroids normal in size. Lymph nodes of neck hyperplastic. Brain normal. Hypophysis thought to be normal in size.

Bones.—Ribs: Can be cut with sharp knife, but offering great resistance. Gross appearance: ribs normally formed. Contour of costochondral junctions normal. On section line of junction of shaft and cartilage sharply defined, but in microscopic preparation examined not quite straight, presenting two broad and shallow notches. Cortex normal thickness. Microscopic: cartilage normal. Zone in which cells have columnar arrangement much less deep than in rib of younger animal. Cartilage cells forming columns and columnar formation normal. Calcium deposition in cartilage normal for older dog. It forms continuous band extending from one side of rib to other, and is present not only between columns of cartilage cells in intercellular substance but also between cells of columns themselves, completely enveloping them. Calcified zone of cartilage continuous with osseous elements of shaft at only two or three points; at those points osseous elements in form of fully developed trabeculae of bone, not calcified columns of intercellular substance undergoing transformation into bone. For greater part of its extent calcified cartilage in contact with normal marrow. No invasion of cartilage by marrow vessels seen, and indeed that part of marrow bordering on cartilage possesses no more capillaries than marrow elsewhere. Trabeculae normal. Not even thin rim of osteoid visible anywhere.

Femur: So hard it can be cut only with saw. Gross: normal contour. Section of lower end: fusion of epiphysis and diaphysis has occurred. Articular cartilage has been reduced to thin film. Cortex is 1.5 mm. in thickness and is exceedingly sharply demarcated. Microscopic: Finer structure of bone exactly like that described in detail in case of dog 40, except that not even the rim of normal osteoid seems to be present (the section is stained so deeply with eosin that this might be obscured). Section of upper end of femur: gross and microscopic appearances absolutely normal. (Staining excellent.) No osteoid demonstrable.

NECROPSY, 39, CONTROL, FEB. 5, 1915.—Body of normal dog. On primary incision considerable fat. Thymus large, weighing, with some fat, 5.1 gm. Suprarenals do not appear to differ very much from those of two thymectomized animals in respect to width of medulla. All other organs normal.

Bones.—Ribs and femur (lower end), gross and microscopic: Findings correspond exactly to those given under dog 39, except that many of thicker trabeculae of rib, and some of trabeculae of femur, have a distinct osteoid border. This latter considerably more marked than that seen in dog 40, but probably does not exceed the normal for dog.

NECROPSY, DOG 40, FEB. 12, 1915.—Well nourished dog. Normal frame. Contour of limbs normal. Hair normal. On primary incision considerable fat. Silk stitches mark site of operative wound. No inflammatory process present. Shape of thorax normal. On opening latter and dissecting along great vessels of neck and about thyroids no tissue suggesting thymus found except small piece behind right innominate vein saved for serial section; probably lymph node. Lungs normal. Heart normal. Spleen normal, showing no signs of hyperplasia. Suprarenals normal. Impression obtained that cortex is a little broader than commonly found. Kidneys normal. Pancreas normal. Stomach and intestines normal. Liver normal. Testes normal. Brain and hypophysis normal.

Bones.—Ribs: Can be split with sharp knife, but are hard. Gross appearance: Contour of costochondral junction normal. On section junction of cartilage and shaft clean cut, forming approximately straight line at right angles to shaft. Cortex normally thick. Microscopic: Cartilage normal. Columns of cartilage cells parallel. Calcium deposition in cartilage normal. Trabeculae in shaft normal. Along some of trabeculae thin, osteoid border is seen. Marrow normal.

Femur: Normal contour, strong. It is necessary to cut with a saw. Lower end of femur, gross: on anteroposterior section of lower end found that epiphyseal cartilage no longer exists, fusion of epiphysis with diaphysis having taken place. Cortex has normal width, estimated at about 2 mm. Articular cartilage has normal thinness of the adult bone. Microscopic: Epiphyseal portion of bone distinguished from diaphyseal by greater fat content of marrow and different direction of trabeculae. Marrow in that portion of bone very largely replaced by fat; trabeculae normal. No sign of osteoid. In diaphyseal portion trabeculae also entirely normal, showing here and there thinnest rim of osteoid, a condition strictly normal for dog. Marrow contains considerable fat, but much less than in epiphyseal portion. Number of osteoclasts usually found in normal bone visible; some round giant cells scattered through marrow. Upper end of femur, microscopic: section shows normal condition.

NECROPSY, DOG 40, CONTROL, JULY 10, 1913.—Since the dog was a control, necropsy was regarded as unimportant, and no description made. Specimens of ribs, femur and organs of internal secretion saved in formalin for possible reference.

Bones.—Ribs and lower end of femur normal. Thicker trabeculae in rib, and trabeculae in femur show here and there the exceedingly thin border of osteoid described as present in dog 40. Fusion of epiphysis and diaphysis of lower end of femur in section examined has already taken place.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 8

Suprarenals.—Medullae in numerous sections taken from various parts of adrenals of dogs 39, 39, control, and 40, approximately equal in width at their widest points. No histologic differences noted, except considerably more fat in medulla of dog 39, control, than in medulla of dogs 39 and 40.

Pancreas.—Normal in all three animals.

Spleen.—Section of spleen of dog 40 missing. Those of other two dogs show microscopically prominent malpighian bodies, those in 39, control, being the larger. In both animals they show central areas composed of larger, more loosely arranged cells with paler nuclei; in these central areas are degenerated cells, nuclear fragments, etc. In dog 39, control, evidences of cell destruction are most marked. Proliferation of endothelial cells gives centers of many malpighian bodies pink color.

Hypophysis.—Normal in all three animals, showing no differences.

Parathyroids.—Normal.

Thyroids.—Thyroid of dog 40 composed of acini of all sizes, predominantly large ones. Epithelium in general very thin, flat; even nuclei are flat. An abundance of colloid which takes on a rather pale pink stain. There is one rather circumscribed area in which acini are smaller and epithelium more cuboidal. No hyperplasia. Thyroid of dog 39 is that of simple, normal appearing gland, in which there is a moderate amount of colloid with evidence in places of newly growing tissue. Other than this nothing of special note. That of dog 39, control, presents a simple colloid picture in which the acini are slightly larger than normal. Colloid stains rather bright pink. Epithelium flattened; in certain areas aggregations of smaller acini, but no infolding anywhere to be seen. No abnormalities otherwise.

Serial sections of tissues for thymus rests from dog 40 failed to show the presence of any. Every fourth section examined, using mechanical stage. Serial sections taken from dog 39 were accidentally not examined.

TABLE 15.—Body Weights and Weights of Organs of Litter 8

	Body Weight, Gm.	Heart, Gm.	Spleen, Gm.	Liver, Gm.	Kidneys, Gm.	Supra-renals, Gm.	Thy-roids, Gm.	Testes, Gm.
Dog 39*	4,308	37.5	8.0	194.0	30.4	0.85	0.5	3.8
Dog 40†	6,122	43.0	16.0	216.0	39.0	0.8	0.9	18.0
39 control†	6,689	53.0	12.5	257.0	30.6	0.91	0.65	13.6
40 control‡								

* Sick when killed. † Healthy. ‡ Died of distemper; weights not recorded.

Experiment 9.—Litter 9, composed of two dogs of mongrel stock, 43 and 43, control; mother a terrier; born Feb. 9, 1914.

TABLE 16.—Experimental Data of Litter 9

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
43	M	20 days	2 mo. 18 days	1 mo. 27 days	Pyemia from mange
43C	M	.....	2 mo. 18 days	1 mo. 27 days	Pyemia from mange

March 1, 1914: Operation on dog 43, 20 days old. Thymus, 3.2 gm., thought to be completely removed. Postoperative condition good. Weight: dog 43, 765 gm.; control, 875 gm.

March 17: Dog 43 in fine condition. Wound healed. Weight: dog 43, 1,100 gm.; control, 1,290 gm.

March 31: Dog 43 well nourished and vigorous; shows signs of mange. Abscess has appeared in midsternal region. No changes noted in skeleton or gait. Dog 43, control, similarly affected by mange. Roentgenograms of extremities of both animals show normal condition. Weight: dog 43, 1,162 gm.; control, 1,728 gm.

April 13, 1914: Both animals in fair condition in spite of mange. Control heavier. Roentgenograms again show normal condition. Weight: dog 43, 1,270 gm.; control, 1,850 gm.

April 20: Both animals thin. Mange has become universal. Dog 43 has corneal ulcer and dog 43, control, abscess on left hind leg. Weights not taken.

April 27: Both animals died.

Necropsy, Dog 43, April 28, 1914.—Body greatly emaciated. Superficial abscess immediately underneath skin on left side of thorax. No subcutaneous fat. On opening thorax and dissection of great vessels of neck, and in region of thyroids, no thymus tissue found. Fat from anterior mediastinum removed for study for thymus rests in serial section. On dissection of neck retropharyngeal abscess situated at level of larynx contained about 1 c.c. pus. Heart normal. Lungs normal. Spleen small; malpighian bodies not prominent. No abnormalities noted in other organs.

Bones.—Ribs: Cut easily with razor. Gross appearance: very slender bone. Costochondral junctions normally shaped. On section line of junction of cartilage and shaft well defined, straight. Cortex thin. Microscopic: Cartilage cells arranged in parallel columns; appearance normal. Column deposition normal. Columns calcified; intercellular substance parallel and numerous. New bone formation normal, but later first becomes very plain well down in diaphysis. Trabeculae normal. No sign of osteoid. Periosteal bone formation normal.

Tibia, lower end: Cuts readily with razor. Gross appearance: Small bone. Proliferative zone of cartilage separating shaft from nucleus of ossification of lower end of epiphysis about 1 mm. wide. Line of separation of cartilage from shaft absolutely definite. Cortex normal thickness. Microscopic: Endochondral ossification absolutely normal. Deposition of calcium in cartilage regular, uniform throughout. No osteoid except thin rim just visible along occasional trabecula well away from epiphyseal-diaphyseal junction. Tibia upper end: Absolutely normal.

NECROPSY, 43, CONTROL, APRIL 28, 1914.—Description of necropsy findings omitted. Thymus showed most extreme atrophy. No Hassall's corpuscles anywhere. Bones normal except for marked degree osteoporosis. Ribs and femur examined.

Notes on microscopic findings in spleen and organs of internal secretion of dogs 43 and 43, control, omitted because of postmortem alterations.

Serial sections of tissues from thymectomized dog 43 examined for thymus rests failed to show the presence of any. Every fourth section examined using mechanical stage.

EXPERIMENT 10.—Litter 10, composed of four mongrels, dogs 60, 60, control, 61 and 61, control, born Nov. 22, 1912; dog 61 a male, other three females.

TABLE 17.—EXPERIMENTAL DATA OF LITTER 10

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
60	F	18 days	3 mo. 5 days	2 mo. 17 days	Ascaris infection; diarrhea
60C	F	.....	3 mo. 7 days	2 mo. 17 days	Ascaris infection; diarrhea
61	M	18 days	3 mo. 1 day	2 mo. 13 days	Ascaris infection
61C	F	.....	2 mo. 15 days	1 mo. 27 days	Ascaris infection; diarrhea

Dec. 10, 1914: Operation on dog 60, aged 18 days. Removal of thymus thought complete. Weight, 1.52 gm. Operation also on dog 61. Weight: thymus, 2.4 gm. Postoperative condition of both animals good. Weight: dog 60, 735 gm.; control, 740 gm.; dog 61, 751 gm.; control, 580 gm.

December 22: Wounds thymectomized dogs clean. Stitches removed. Weight: dog 60, 800 gm.; control, 1,180 gm.; dog 61, 870 gm.; control, 1,150 gm. Roentgenograms of extremities of all four animals taken, revealing no abnormalities.

Jan. 10, 1915: No changes found on examination of thymectomized animals. No disturbance in gait or diminished rigidity of extremities. Both dogs 60 and 60, control, thin, 60 more so that 60, control. Other two animals remain normal. Roentgenograms again taken show no abnormal condition. Weight: dog 60, 1,000 gm.; control, 1,130 gm.; dog 61, 890 gm.; control, 1,120 gm.

Feb. 2, 1915: Dogs 60, 60, control, and 61, control, appear to be losing weight, 61, control, the most rapidly. Have some diarrhea, and are thought to have ascaris infection. Neither thymectomized animal has shown any change attributable to loss of thymus. Roentgenograms taken once more fail to reveal any abnormal condition of bones. Weight: dog 60, 910 gm.; control, 1,070 gm.; dog 61, 890 gm.; control, 920 gm.

February 6: Dog 61, control, died.

February 18: Three remaining dogs thin; two thymectomized animals show no symptoms not shared by control. Weight: dog 60, 1,010 gm.; control, 1,245 gm.; dog 61, 1,900 gm.

February 23: Dog 61 died.

February 27: Dog 60 died.

March 1, 1915: Dog 60, control, died.

NECROPSY, DOG 60, MARCH 1, 1915.—Much emaciated body. Hair normal. Legs show no deformities. On primary incision no fat. No tissue suggesting thymus either in thorax or in course of large vessels, or in neighborhood of thyroids; all tissue possibly thymus collected and saved for serial section. Heart normal. Spleen light red; cut surface dry; malpighian bodies scarcely visible. Suprarenals normal, but appear somewhat flatter than usual. On section medulla unusually thin. Kidneys normal. Pancreas normal. Liver normal. Ovaries normal. Hypophysis normal. Brain normal.

Bones.—Ribs: cut easily with razor. Gross appearance: slender. Shape of costochondral junctions normal. On section junction of shaft and cartilage forms clear cut straight line. Cortex thin. Microscopic: Cartilage arranged in rather long parallel columns. Calcium deposition normal; everywhere the same. Columns of calcified intercellular substance in shaft adjacent to cartilage normal, having strictly parallel arrangement. New bone formation normal. No sign of osteoid. Rarefaction marked.

Femur: Cuts with razor with little resistance. Gross appearance: zone of proliferating cartilage ending in shaft about 1 mm. thick. Absolutely distinct transition from cartilage to shaft. Cortex thin. Microscopic: Endochondral ossification absolutely normal, not showing slightest irregularity. Calcium deposition normal. No trace of osteoid. Rarefaction marked.

NECROPSY, 60, CONTROL, MARCH 1, 1915.—Much emaciated dog. On incision of thorax thymus represented by thin film tissue weighing approximately 0.35 gm. Spleen light red; malpighian bodies not visible. Kidneys normal. Pancreas normal. Liver normal. Ovaries normal. Suprarenals flat; cortex broader than medulla, latter represented in section taken from several parts of organ by mere line.

Bones.—Ribs and femur, lower end, gross and microscopic: except both bones offered more resistance to cutting than in case of thymectomized dog, and cortex of femur seemed thicker than cortex of dog 60, no differences noted.

NECROPSY, DOG 61, FEB. 23, 1915.—No thymus rest found. All likely tissue in neck and thorax saved for serial section. Suprarenals show normal relationship between medulla and cortex. No abnormalities observed in other organs.

Bones.—Ribs: can be easily split longitudinally with razor. Gross appearance: slender. Shape costochondral junction normal. On section line of meeting of cartilage and shaft sharp and straight. Cortex thin. Microscopic: Cartilage normal. Cells arranged in parallel columns. Calcium deposition in preparatory zone of ossification normal. Trabeculae bone normal. Narrow zone osteoid can be seen in a few places along trabeculae. Marrow normal.

Femur: Splits longitudinally with razor with great ease. Gross appearance: external configuration normal. On section of lower end nucleus ossification of epiphysis large or small, depending on plane through which section passes. So also, line of junction comparatively straight or undulating, depending on plane of section. Zone of cartilage of epiphysis bordering on shaft about 1 mm. thick. Line of separation of cartilage and shaft clearly defined. Cortex thin. Microscopic: Cartilage cells of proliferative zone beautifully arranged in parallel columns. Calcium deposition in cartilage absolutely normal.

NECROPSY, DOG 61 CONTROL, FEB. 6, 1915.—Body much emaciated; skeleton outlined through skin. No subcutaneous fat. On opening thorax thymus mere film enveloping auricle and left innominate vein. Except for few round worms upper part of small intestine, and some changed blood, no abnormalities.

Bone.—Gross and microscopic: rib and femur correspond to rib and femur of dog 61 in almost all particulars. Thought, however, femur dog 61, control, cuts with less resistance than femur dog 61.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 10

Spleens of all four animals showed extreme degrees of atrophy.

Suprarenals.—Medullae of suprarenals of two thymectomized dogs as broad as medullae of control animals. Medullae of two thymectomized dogs studied on account of postmortem degeneration. Architecture of cortex and morphology of cortical cells same in suprarenals of thymectomized animals as control animals.

Thyroids of thymectomized dogs showed such extensive degenerative changes (swelling, desquamation of epithelium) as to render them unfit for histologic study. Thyroids of control dogs characterized by marked variations in size of alveoli. Some parts of sections examined appeared almost solid. Large alveoli contained colloid in abundance; small alveoli none. Alveolar epithelium cuboidal. No infoldings.

Hypophyses.—Normal.

Parathyroids.—Normal.

Thymus glands dog 60, control, and 61, control, showed extreme degrees of atrophy.

Serial sections of tissues for thymus rests from thymectomized dogs 60 and 61 failed to show presence of any. Sections 15 microns thick. Every fourth section examined using mechanical stage.

EXPERIMENT 11.—Litter 11, composed of 62 and 62, control, born Dec. 3, 1914. Mongrels, 62, male; 62, control, female.

TABLE 18.—EXPERIMENTAL DATA OF LITTER 11

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
62	M	20 days	12 mo. 21 days	12 mo. 1 day	Killed with ether
62C	F	.....	3 mo. 21 days	3 mo. 1 day	Killed accidentally by drowning*

* Animal, blind as result of congenital cataract, fell in well. Body not recovered, no necropsy.

Dec. 23, 1914: Operation on dog 62, 20 days old. Thymus apparently completely extirpated. Operation well borne. Weight: thymus, 2.4 gm. Dog 62, control, has congenital visual defect, making him almost completely blind. Weight: dog 62, 793 gm.; control, 567 gm.

Jan. 2, 1915: Weight: dog 62, 1,520 gm.; control, 1,530 gm.

January 4: Operative wound healed; stitches removed. Condition operative animal good. Weight: dog 62, 1,000 gm.; control, 960 gm.

January 12: Roentgenograms of extremities both animals show normal condition.

January 27: Thymectomized dog excellent condition. Gait normal. No abnormalities in bones of extremities can be found. Weight: dog 62, 1,480 gm.; control, 1,410 gm.

Feb. 2, 1915: Dog 62 normal. Weight: dog 62, 1,520 gm.; control, 1,530 gm.

February 17: Dog 62 fine example of active, healthy, normal dog, showing absolutely no disturbance in gait, no enlargement of ends of long bones of legs or bending. Hair and teeth normal. Appetite normal: Weight: dog 62, 2,345 gm.; control, 2,050 gm.

February 25: Roentgenograms show no changes in bones of extremities of dog 62.

March 3, 1915: Dog 62 well nourished; continuing to outweight control. No changes detected. Weight: dog 62, 3,080 gm.; control, 2,690 gm.

March 24: Dog 62, control, drowned by falling in well. Body not recovered.

March 30: Animal now fully grown, extremely active; apparently intelligent animal; remains normal. Roentgenograms of extremities show no changes. Weight: dog 62, 4,110 gm.

April 20, 1915: Roentgenogram of legs still does not reveal slightest changes. Weight: dog 62, 4,240 gm.

May 3, 1915: Dog 62 continues in all respects normal. Not faintest suggestion of bone change. Appetite normal. Intelligence normal. Roentgenograms of extremities normal. Weight: dog 62, 6,803 gm.

Dec. 22, 1915: No record of weight kept of dog 62 after May 3 until animal's death because of loss of basis of comparison in death of control. Dog 62 remained healthy, normal dog, however, developing no changes of any sort. On this date killed with ether. Postmortem weight, 6,803 gm.

NECROPSY, DOG 62, DEC. 22, 1915.—Excellently nourished dog; limbs perfectly formed; thorax normal contour. Hair normal. Silk ligatures found marking site of incision. Abundant subcutaneous fat. On opening thorax upper lobe of left lung slightly adherent. Just above left innominate vein small loose mass tissue somewhat resembling thymus. (Frozen section shows it is fat.) Small piece of tissue which it is thought may be thymus removed from behind left innominate vein. (Frozen section shows this to be fat also.) Heart normal. Lungs normal. Spleen small; malpighian bodies normally prominent. Suprarenals normal. Relation between cortex and medulla thought normal. Pancreas normal. Liver normal. Stomach and intestines normal. Lymphoid elements of intestine not hyperplastic. Hypophysis normal.

Bones.—Ribs, gross examination: contour ribs normal. Costochondral junctions not enlarged. On section ribs through costochondral junction bone cuts with normal resistance. Line junction of cartilage and shaft clear cut. Cortex normal thickness. Microscopic: Cartilage normal. Cells bordering on shaft arranged in parallel columns. Calcium deposition in cartilage normal. Trabeculae shaft normal. Thin border osteoid seen along some of trabeculae. Bone marrow normal (Fig. 27).

Femur: Gross examination: femur has normal contour; is strong bone. On section with saw seen that fusion between epiphysis and diaphysis of lower end of femur has taken place. Microscopic: Trabeculae show no osteoid border except perhaps thinnest possible line present here and there. Marrow largely replaced by fat.

NOTES ON FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOG 62, LITTER 11^{200}

200. Weights of organs not recorded.

Spleen shows considerable atrophy; malpighian bodies poorly developed, seen with difficulty. Pulp cells diminished. Trabeculae extremely thick and numerous.

Suprarenals.—Normal. Architecture of cortex and morphology cells absolutely normal. Medulla also appears normal. In stained specimen medulla has about same width as cortex.

Pancreas.—Normal.

Hypophysis.—Appears normal.

Parathyroids.—Not examined.

Thyroid.—Thyroid presents absolutely normal appearance. Alveoli markedly uniform in size and filled with colloid. Lining epithelium cuboidal. No infolding. No desquamation of epithelium.

Testis.—Testis shows all stages of spermatogenesis, completely developed, normal organ.

Serial sections of tissues for thymus rests from thymectomized dog 62 failed to show presence of any.

EXPERIMENT 12.—Litter 12, composed of dogs 67 and 67, control, mongrels, born Jan. 10, 1916.

TABLE 19.—EXPERIMENTAL DATA OF LITTER 12

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
67	M	15 days	3 mo. 3 days	2 mo. 19 days	Died after acute illness following exposure
67C	M	.....	3 mo. 3 days	2 mo. 19 days	Killed (with ether) but sick with same infection as that which killed dog 67

Jan. 25, 1916: Operation on dog 67, 15 days old. Thymus thought taken out in entirety; weight, 1.7 gm. Neck dissected up to thyroids. Weight: dog 67, 580 gm.; control, 660 gm.

Feb. 10, 1916: Thymectomized animal normal. Wound healed. Control somewhat larger; thymectomized dog more active. Weights not taken.

March 8, 1916: This pair of animals kept as pets at private house. Dog 67 quite as lively as mate. No change in hair, contour or rigidity of leg bones or gait. Keeper of animals thinks control has greater appetite, but operative animal more intelligent. Weight: thymectomized animal, 2,160 gm.; control, 2,680 gm.

April 12, 1916: Both animals remained normal until three days previous to present note, when they were exposed to severe cold (left out accidentally over night). Both animals developed respiratory infections; control only slightly affected; thymectomized animal, taken acutely ill, died on above date. Control killed with ether on following day. Weight: dog 67, 2,821 gm.; control, 4,445 gm.

NECROPSY, DOG 67, APRIL 13, 1916.—Poorly nourished dog; well formed. No deformity extremities. Stitch abscess present in line of operative wound, containing drop or two of pus. Thorax shows pigeon breast deformity. Some subcutaneous fat present. Lungs adherent to sternum in region of incision. No thymus found anywhere; to tissues resembling thymus. All fat, cellular and lymphatic tissues which lay in anterior mediastinum about innominate vein, in neck along great vessels and about thyroid gland, dissected out in toto and saved for serial section. Upper part of fat overlying pericardium removed also for serial section Thus, all tissues in regions normally occupied by thymus removed for serial section. Both pleural cavities free. Surface both lungs covered by petechial hemorrhages more or less scattered. Small area of pneumonia in left lung. Heart normal. Right thyroid measures 2.1 by 0.6 by 0.5 cm.; left, 1.9 by 0.8 by 0.4 cm. Both appear normal. Parathyroids usual size. Abdominal subcutaneous fat fairly abundant. Peritoneum normal. Spleen dark red; malpighian bodies so small as to be barely visible; cut surface has dark red homogeneous color. Left suprarenal measures 1.7 by 1 by 0.4 cm.; right suprarenal measures 1.8 by 0.8 by 0.3 cm. Cortex both suprarenals about 1 mm. thick; medullae certainly not increased in thickness, in narrowest parts measuring about 0.5 mm. and in widest about 1.6 mm. Medulla thickest where cross section of suprarenal has more rounded shape. Pancreas normal. Kidneys normal. Liver normal. Testes normal. Hypophysis normal. Intestinal tract contains few worms and considerable amount of mucus and congested areas in region of middle and lower parts of small intestine, but shows no ulceration. Lymphatic elements not hyperplastic.

Bones.—Ribs, gross appearance: costochondral junctions seen from inner side thorax appear slightly abnormal, particularly lower ones. They form slightly larger swellings than normal, and appear highly vascularized. On section knife slips through easily, encountering considerable resistance only after it enters shaft of rib at a centimeter or so distance from cartilage. That

part of shaft which borders on cartilage has pale appearance. Junction of cartilage and shaft form, however, approximately straight line. Cortex about normal width (Fig. 33). Microscopic: Near shaft cartilage cells arranged normally in parallel columns, and have normal appearance. Deposition of calcium irregular. In several places for distances equal to breadth of three to seven cartilage columns, calcium deposition wanting; elsewhere in cartilage appears essentially normal. Marrow vessels invading cartilage columns slightly in advance of others at points in cartilage where calcium deposition fails, so that line of junction of cartilage and shaft as seen under microscope more irregular than normal. Columns of calcified intercellular substance adjacent to cartilage numerous, parallel, showing beginning bone formation along sides, and have normal appearance. Short distance away from cartilage border distinctly pathologic condition presents itself in form of bandlike zone, free from calcium which, not more than two to three cartilage cells wide, traverses shaft at right angles, more or less paralleling line of junction of cartilage and shaft; does not extend continuously from one side of shaft to other, however, for at two or three points normal calcified columns of intercellular substance cross it. Where these do not cross, i. e., where they end abruptly on one side of it and begin again on other, is frequently found that cartilage or small groups of cartilage cells, having more or less columnar arrangement, and lying in line with calcified columns, fill in gaps. These groups of cartilage cells are not only free from calcium deposition, but have appearance of being compressed and show signs of degeneration. Stain pink with eosin. Farther down in shaft osteoid borders visible here and there along some of trabeculae. Though not so broad or so numerous as those seen in rib of dogs 71 and 75, control, they are in places exceedingly well marked. They occur, with a few exceptions, only along thicker trabeculae, and quite uniformly on one side or at single point along one side. Many of larger trabeculae do not show any border of osteoid. Apparently some rarefaction of trabeculae is present. Marrow cells greatly diminished in numbers.

Femur, gross appearance: On section through lower end cartilage has wavy outline, but appears to be same thickness everywhere. Below cartilage, however, is zone about 5 to 6 mm. in thickness of yellowish white color, thought due to presence of fibrous tissue. Cortex of bone of about normal thickness; saw easily traverses shaft of bone. Nucleus ossification of epiphysis large. Zone cartilage separating it from shaft about 1 mm. thick. Microscopic: Cartilage in parallel columns. Deposition of calcium in cartilage absolutely normal. Columns of calcified intercellular substance have strictly parallel arrangement, and are close together. New bone formation normal. No trace of osteoid.

Sections from tibia, radius and humerus show no abnormal conditions (Figs. 34 and 35).

Necropsy, Dog 67, Control, April 14, 1916.—Animal well nourished, much larger and heavier built than dog 67. Subcutaneous fat more abundant. Thymus has reddish color; does not extend into neck. Weight: 3 gm. Spleen resembles that of dog 67. Suprarenals normal. Medulla has about same thickness as medulla of thymectomized mate of animal, certainly not narrower. Intestines show hyperplasia lymphoid elements in contrast to condition in thymectomized dog; all other organs normal.

Bones.—Ribs, gross appearance: Costochondral junctions of ribs externally appear normal. On section junction of cartilage and shaft seems to be highly vascularized. Same witish zone visible in case of thymectomized animal immediately adjacent to cartilage present in this control, but not quite so wide. Rib easily cut with knife. Microscopic: Normal. Older trabeculae of bone somewhat removed from costochondral junction, show here and there rim of osteoid almost but not quite so prominent as that found in thymectomized animal.

Femur, gross and microscopic: Normal; exactly like femur of dog 67.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 12

Spleen.—Spleen of dog 67 shows considerable atrophy; malpighian bodies small. On microscopic examination found to contain many nuclear fragments and degenerated cells. Spleen of dog 67, control, not atrophic. Malpighian bodies appear considerably larger than thymectomized dog.

Suprarenals.—Suprarenals of dog 67 and 67, control, show no essential differences.

Hypophyses.—Normal in both animals.

Pancreas.—Normal.

Parathyroids.—Not examined.

Thyroid.—Thyroid of dog 67 splendid example of normal organ. Structure of gland extraordinarily uniform; colloid abundant. Epithelium lining alveoli cuboidal. No infolding; no exfoliation. Thyroid 67, control, shows so much exfoliation and swelling of alveolar epithelium that its study is useless.

Testes.—Testes of two animals show no differences; both in embryonic stage of development.

Thymus.—Thymus of dog 67, control, has begun to involute. Few small Hassall's bodies can be seen.

Serial sections of tissues for thymus rests from thymectomized dogs 67 and 67, control, failed to show presence of any. Sections 15 microns thick. Every fourth section examined using mechanical stage.

TABLE 20.—BODY WEIGHTS AND WEIGHTS OF ORGANS OF LITTER 12

	Body Weight, Gm	Heart, Gm.	Liver, Gm.	Kidneys, Gm.	Suprarenals, Gm.	Thyroids, Gm.	Thymus, Gm.	Testes, Gm.
Dog 67.....	2,821	26.0	140	25.2	0.61	0.71	...	0.7
67 control.....	4,445	38.5	303	48.0	0.7	2.9	3.0	0.9

EXPERIMENT 13.—Litter 13, composed of dogs 70 and 70, control, both females, born Feb. 16, 1916, mongrel stock; mother pure blooded terrier.[201]

TABLE 21.—EXPERIMENTAL DATA OF LITTER 13

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
70	F	15 days	6 mo. 2 days	5 mo. 17 days	Killed with ether; sick
70C	F	.....	3 mo. 20 days	3 mo. 5 days	Died, probably as result of infection with round worms*

* When these two animals were four weeks old they were sent to Laboratory Janitor D. to be cared for at his home. This fact is probably of some importance in explaining their illnesses and the death of 70 control. Almost all the animals cared for by D. became sick and died (see Footnote 208).

March 1, 1916: Operation on dog 70, 15 days old. Incision not carried so far down thorax as usual, but upward until both thyroids and parathyroids were exposed. Removal of thymus thought complete, but not with so much certainty as in previous operations, for reasons of shortness of incision in thorax, which was carried only little below base of heart. Thymus did not extend up into neck. Weight: thymus, 2.3 gm. Postoperative condition of dog good. Weight: dog 70, 660 gm.; control, 510 gm.

March 9: Thymectomized animal in fine condition, showing little effect from operation. Weight: dog 70, 975 gm.; control, 782 gm.

201. Although animals were of same litter, there was greatest difference in their appearance.

March 21: Dog 70 shows no abnormalities. More active and stronger looking animal than control. Weight: dog 70, 1,350 gm.; control, 1,145 gm.

March 29: Animals put under care of laboratory janitor D. at his home, where there is small yard.

April 20, 1916: Thymectomized dog still shows no changes; active, apparently intelligent, and has no disturbance in gait, no thickening or diminished rigidity of long bones. Thymectomized dog growing more rapidly than control; latter looks unhealthy, and is thought to be infected with worms. Weight: dog 70, 2,130 gm.; control, 1,350 gm.

June 6, 1916: Since last note dog 70, control, has become emaciated. Numerous treatments for ascaris infection failed to cause improvement. Diarrhea. On this date dog 70, control, died. Dog 70, on other hand, continues in perfectly normal condition from every standpoint.

Aug. 18, 1916: Dog 70 killed with ether when in moribund condition; had been acutely sick for one week; sudden onset. Nature of illness not clear. Vomited all food as soon as given, even water, and emaciated rapidly. It is thought that animal had acidosis. He had been given by D. numerous doses of vermifuge, and perhaps had been poisoned by overdose.

NECROPSY, DOG 70, CONTROL, JUNE 6, 1916.—Body much emaciated. No subcutaneous fat. On opening thorax atrophied thymus found. Spleen atrophic, showing no malpighian bodies. Stomach filled with mucoid fluid. In duodenum just beyond pyloric ring two ulcers, about 4 to 5 mm. in circumference, which extend certainly to muscular layers, if not into them. Jejunum and ileum normal except that lower part of ileum contains mucoid fluid blood tinged in places; mucous membrane congested along rugae without showing ulcerations. Colon contains mucoid, blood stained fluid. Adrenals appear normal. Other organs show nothing noteworthy.

Bones.—Microscopic examination of both ribs and femur shows normal conditions.

NECROPSY, DOG 70, AUG. 18, 1916.—Emaciated body. Subcutaneous fat scant. No inflammatory process about line of incision. No thymus rests found in thorax or along vessels in neck or about thyroids. All fatty and lymphoid tissues dissected away from those regions and saved for examination of thymus rests by serial section. All organs appeared normal except that larger of them were covered with small yellow spots exactly like those described in case of dogs of litters 2 and 3.[202] Medullae of suprarenals did not appear wider than normal. Gastro-intestinal tract showed nothing abnormal.

Bones.—Ribs, gross examination: Contour normal. Costochondral junctions not enlarged. On section bone cuts with normal resistance. Line of junction between cartilage and shaft normal. Cortex of normal thickness. Microscopic: Cartilage normal. Cartilage cells bordering on shaft arranged in parallel columns. Calcium deposition normal. Shaft normal. Trabeculae show in places normal, thin, osteoid border (Fig. 23).

Femur, gross and microscopic: Normal. Proliferative cartilage bordering on shaft arranged in narrow, parallel columns. Calcium deposition absolutely uniform. Trabeculae normal. Marrow normal, except that several small abscesses can be found, some of which contain dead bone.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOG 70 OF LITTER 13

Spleen.—Presents fairly normal structure. Malpighian bodies large and show centrally pale-staining areas. In these areas numerous nuclear fragments. Pulp appears normal.

Suprarenals.—Appear absolutely normal. In stained specimens medulla appears to have about width of cortex; medullary cells appear normal. Architecture and cells of cortex appear absolutely normal.

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202. See footnote 195.

Pancreas.—Normal.

Parathyroids.—Normal.

Thyroid.—Normal. Alveoli remarkably uniform. Colloid abundant. Alveolar epithelium cuboidal, in places cylindrical. No desquamation; no infolding.

Serial sections of tissues for thymus rests from dog 70 failed to show presence of any. Every fourth section examined, using mechanical stage.

TABLE 22.—Body Weights and Weights of Organs of Litter 13

	Body Weight, Gm.	Heart, Gm	Spleen, Gm.	Liver, Gm.	Kidneys, Gm.	Suprarenals, Gm.	Pancreas, Gm.	Thyroids, Gm.	Thymus, Gm.
Dog 70.....	3,600	25.9	6.6	137	29.0	0.9	13.3	0.82	...
70 control....	1,330	13.2	1.9	90	17.3	0.5	5.6	0.21	0.6

Experiment 14.—Composed of seven mongrel puppies, mixture of collie and spaniel, born April 10, 1916. Dogs 71, 75 and their control, 71 and 75, control, (Group A) males; 72, 73, 74 and their common control 72 and 73, control, (Group B) females.[203]

TABLE 23.—Experimental Data of Dogs Used in Experiment 14

Dog	Sex	Age at Operation	Age at Death	Period of Observation	Cause of Death
			Group A		
71	M	13 days	1 mo. 19 days	1 mo. 6 days	Died after acute illness with sudden onset, 3 days duration
75	M	13 days	2 mo. 9 days	1 mo. 26 days	Died (progressive emaciation and diarrhea)
71-75C	M	.....	2 mo. 13 days	2 mo. 1 day	Killed (sick)
			Group B		
72	F	13 days	2 mo. 9 days	1 mo. 26 days	Died (emaciation and diarrhea)
73	F	13 days	1 mo. 20 days	1 mo. 7 days	Sacrificed (with ether) on onset of acute illness
74	F	13 days	1 mo. 28 days	1 mo. 15 days	Died (abscessed chest wall; diarrhea)
72-73-74C	F	.....	2 mo. 13 days	2 mo. 1 day	Sacrificed (with ether). Apparently in fairly healthy condition

Group A.—April 23, 1916: Operation on dogs 71 and 75, 13 days old; well borne by both. Removal of thymus from both animals appeared complete. Thymus from dog 71 weighed 2.11 gm.; from dog 75, 2.31 gm. Weight: dog 71, 820 gm.; dog 75 and control not taken until June 28; on that date dog 75 weighed 870 gm.; control, 940 gm.

May 5, 1916: Stitches removed from both dogs 71 and 75. Both had small superficial stitch abscesses; contents evacuated. Health of dog 71 appears in no wise affected by abscess; dog 75 looks sick.

May 29: Dog 71 died. Suddenly taken sick in night, howling as if in pain, and died two days later. Previous to acute illness he had seemed to be doing fairly well, though he was a little smaller than his own control animal, and considerably smaller than the female control in group B. Weight after death, 1,330 gm.

June 19, 1916: Dog 75 died. Had not thriven since operation, had remained smallest of all the animals of the two groups. Did not become acutely sick like dog 71, but had diarrhea, emaciated and died. Weight after death, 980 gm.

203. These animals, together with dogs of litter 13, were put in care of laboratory janitor D. at his home, where feeding and care accorded to animals was exceedingly poor. D. gave animals of this litter a patent vermifuge almost daily. A number of them developed diarrhea. Food which he provided was altogether unsuitable.

June 23: Control of this group killed with ether. He too had become malnourished, but did not seem to be acutely sick. Weight after death, 1,507 gm.

NECROPSY, DOG 71, MAY 31, 1916.—Necropsy performed six hours after death. Poorly nourished body. On primary incision no subcutaneous fat. Small abscess containing drop of pus found at upper part of sternum; otherwise no evidence of any infection. No evidence of thymus found unless possibly beneath left innominate vein. This tissue, as well as all other tissues which might possibly resemble thymus, from thyroid gland to pericardium, dissected away for serial section. Lungs normal. Spleen normal. Heart normal. Malpighian bodies not visible, cross section pink. Suprarenals normal. Cortex about 1 mm. in thickness; medulla about same thickness in widest point. Pancreas apparently normal, though considerable postmortem decomposition has taken place. Entire gastro-intestinal tract thought normal, though considerable postmortem change has taken place; very few round worms present. Kidneys normal. Liver normal. Testes normal. Brain normal. Hypophysis normal.

Bones.—Ribs, gross appearance: Slender; costochondral junction has normal shape. Rib can be split with razor with little resistance. Line of junction cartilage and shaft straight and clearly defined. Cortex thin; marrow pale. Microscopic: Cartilage everywhere normal, near shaft arranged in parallel columns. Calcification of intercellular substance of cartilage bordering on shaft normal. Shaft normal, except that marrow cells appear greatly reduced in number, so that connective tissue framework of marrow is visible; trabeculae unusually thin. No osteoid border to trabeculae anywhere visible. Bordering on cartilage columns of calcified intercellular substance very numerous and have parallel arrangement. Osteoclasts very numerous.

Femur (lower end), gross appearance: Center of ossification of epiphysis well developed; cartilage separating it from shaft about 1 mm. thick. Junction of cartilage with shaft forms clear-cut line, though somewhat curved, depending where section is taken. Cortex thin. Marrow pale. Microscopic: Conditions normal, with following exceptions: Trabeculae not numerous and appear farther apart than normal; osteoclasts present in great numbers, some enormous; along some of trabeculae they are so close together that they form almost continuous line. Marrow cells more numerous than in ribs. No osteoid present anywhere; calcium deposition normal; calcified columns of intercellular substance adjacent to cartilage very numerous, have strictly parallel arrangement, and with their lateral branches form thick, interlacing network. Cartilage normal. Center of ossification of epiphysis normal, except that it contains some islands of necrotic cartilage and shows signs of rarefaction such as already described in the shaft.

NECROPSY, DOG 75, JUNE 19, 1916.—Animal poorly nourished. Small amount subcutaneous fat. Muscles pale. Serous surfaces of thorax and abdominal cavities normal. Over arch of aorta definitely circumscribed, lobulated, light pink mass of tissue suggesting thymus; saved for section; no other tissue resembling thymus found. No evidence of any inflammatory process in region of wound or elsewhere.

Heart normal. Lungs everywhere containing air. Liver pale but apparently normal. Pancreas normal. Suprarenals normal. Spleen light red; no malpighian bodies visible. Intestines congested and contain changed blood and mucus.

Bones.—Ribs, gross and microscopic appearance: Almost identical with those described in case of dog 71. Section through rib at costochondral junction cut somewhat obliquely so that deposition of calcium in zone of cartilage bordering on shaft appears broader than normal, and columns of calcified intercellular substance adjacent to cartilage have in places appearance of cross section of honey comb. Marrow more abundant than in bones of dog 71. Osteoclasts not quite so numerous. Absolutely no sign of osteoid in either rib or femur (Fig. 22).

NECROPSY, CONTROL DOG 71-75 C, KILLED JUNE 23, 1916.—Body poorly nourished. Thymus weighs 0.35 gm. All organs appear normal. No malpighian bodies visible in spleen. In stomach and intestines considerable greenish material consisting of stones and food residue, no parasites discovered.

Bones.—Ribs, gross appearance: Slender; costochondral junctions have normal contour. Shaft can be cut with razor as easily as soft wood. Junction of cartilage and shaft forms slightly irregular line, marked by tiny serrations easily visible to naked eye. Marrow pale. Cortex thin. Microscopic (Figs. 29, 30, 31 and 32): Section slightly oblique with reference to long axis of shaft. Cartilage not normal for as shaft is approached cartilage cells have disorderly arrangement in irregular groups rather than in parallel columns. Many cells which lie close to shaft have rounded or oblong shape with their long axes in longitudinal axis of shaft. Calcium deposition present in cartilage adjacent to shaft, but very irregular. In some places absent altogether, in others fragmentary, and in yet other places normal. At numerous points prolongations from bone marrow seen to extend into cartilage, forming irregular excavations in latter, makinfi cartilage and shaft appear to interdigitate. Prolongations of bone marrow composed chiefly of blood vessels and connective tissue, intermingled with which is much degenerated cartilage. The invasion of cartilage seems to be taking place at points which, for the most part, are not protected by calcium deposition. Cartilage which lies adjacent to shaft fails to stain at many places with hematoxylin, so that it has a light pink color in contrast to the more intense blue of normal cartilage. In one place this degenerated cartilage appears to be undergoing metaplasia into bone. Large island of cartilage showing well marked signs of degeneration present in shaft at some distance from costochondral junction. Columns of calcified intercellular substance in zone of shaft bordering on cartilage quite irregular, doubtless in large part due to somewhat diagonal plane of section. They really do not appear as columns at all, but as short, irregularly shaped masses with notched borders, which in places have an arrangement something like that seen in cross section of honeycomb. Farther down in shaft trabeculae become elongated and parallel to each other. Signs of new bone formation along them are abundant and osteoclasts appear to be as numerous as normal. In many places, however, osteoid instead of bone is present. Borders of osteoid most marked at some distance from cartilage where trabeculae are broadest, and reach a width at some points such as is seen only in advanced rickets or osteomalacia in human beings, but distribution of osteoid border is not universal. Whereas some trabeculae are bordered by it on two sides, in others it appears not at all or on only one side, or at only one point along side or at tip of trabecula. It can be seen on some of the very slender trabeculae near cartilage. Numerous signs of rarefaction present. Marrow appears fairly normal.

Femur (lower end), gross appearance: These do not differ from gross appearances of femora in dogs 71 and 75. Line of junction of cartilage and shaft is clear cut; calcium deposition in cartilage and arrangement of cells in parallel columns absolutely normal. Osteoid border along trabeculae of bone in shaft is only here and there visible, and does not exceed the normal. Osteoclasts in large numbers present.

Group B.—April 23, 1916: Operation on dogs 72, 73 and 74, 13 days old; well borne by all three animals. In each instance thymus seemed to be completely removed. In case of dog 73 thin filament of thymus ascended neck on left side, lying along the great vessels one third of the way to the thyroid. Thymus of dog 72 weighed 1.01 gm.; dog 73, 1.7 gm.; dog 74, 2 gm. Postoperative condition of all three good. Weight: dog 72, 890 gm.; dog 73, 670 gm.; dog 74, 720 gm. (Weight of control not taken until May 28.)

May 28, 1916: All three thymectomized dogs appear to have recovered from operation. They walk about and suckle in almost normal manner.

May 30: Dog 73 suddenly taken sick on evening of May 29. (Dog 71 taken acutely sick with same symptoms May 28.) Began to howl, as if in

pain, and had several convulsions. Janitor killed dog with ether for fear noise would disturb neighbors. Weight: dog 73, taken after death, 1,191 gm.

June 8, 1916: Dog 74 died. Had been sick for a number of days, and become thin and weak. Weight: dog 74, 1,290 gm.

June 19: Dog 72 died. This dog also became progressively weak.

June 23: Control dog killed with ether in comparatively good health.

NECROPSY, DOG 72, DIED JUNE 19, 1916.—(Necropsy performed eight hours postmortem.) Female; moderately well nourished; little subcutaneous fat. Hair and skin normal. No evidence of inflammatory process about site of wound. On removing sternum careful dissection made for thymus rests. Over pericardium is small amount of adiposelike tissue; specimens of this saved for section. Nothing suggesting thymic tissue identified. Pleural surfaces normal. Lungs everywhere crepitant. Heart normal. Abdominal cavity contains small amount straw colored, serous fluid; serous surfaces normal. Spleen normal; no malpighian bodies visible. Kidneys normal. Suprarenals normal. Pancreas normal. Liver normal. Intestinal wall dark red, showing, however, no evidence of necrosis. Intestinal contents consist of relatively small amount of muco-sanguineous material, especially in distal portions. No intestinal parasites.

Bones.—Both ribs and femur present picture described under dog 71 on gross and microscopic examination. No osteoid. Endochondral ossification apparently proceeding in entirely normal manner. Sole pathologic condition is osteoporosis.

NECROPSY, DOG 73, MAY 30, 1916.—Perfectly formed puppy. Hair normal. Bones of legs easily bent, easily fractured; on primary incision subcutaneous fat scant. No vestige of thymus found; all tissues from about thyroid to pericardium which were thought possibly to contain thymus removed and saved for serial section. Lungs adherent to heart, also to sternum. Lungs clear. Heart normal. Spleen normal; malpighian bodies not even visible. Cut surface of spleen homogeneous pink. Small intestines normal. Large mass of worms found in upper part of small intestine. Colon normal except for some portions which showed congestion; no ulceration found. Stomach normal; duodenum normal. Kidneys normal. Suprarenals normal. Cortex of suprarenals about 1 mm. thick; medulla very thin, not much more than line. Ovaries normal. Pancreas normal. Liver normal. Thyroids normal Hypophysis normal. Brain normal.

Bones.—Rib and lower end of radius show only osteoporosis; no osteoid. No disturbance in endochondral ossification. Calcium deposition in cartilage absolutely normal.

NECROPSY, DOG 74, JUNE 8, 1916.—Emaciated animal. On primary incision some fat present. Abscess containing half teaspoonful thick yellow pus found at upper sternal margin, not communicating with chest cavity. Heart normal. Lungs normal. Thyroids normal. Parathyroids normal. Spleen normal; color pink. Malpighian bodies not visible. Kidneys normal. Suprarenals—cortex about 1 mm. in thickness; medulla appears thin, in most places represented by mere line. Pancreas normal. Liver normal. Stomach normal. Stomach contained some bloody fluid together with round worms. Intestinal tract showed unusual amount of congestion most marked in upper half of ileum. Contents of intestine composed of mucus, food and changed blood. No ulceration. Esophagus normal.

Bones.—Ribs and femur show only osteoporosis. Calcification of cartilage normal.

NECROPSY, CONTROL DOG, 72-73-74 C, JUNE 23, 1916.—Thymus weighs 0.35 gm.; has normal, lobulated structure, anterior and lateral to arch of aorta. All organs normal. Gastro-intestinal tract: contents consist of gritty, greenish food residue. No parasites noted.

Bones.—Gross and microscopic examination: Normal except for slight degree of osteoporosis.

NOTES ON MICROSCOPIC FINDINGS IN SPLEEN AND ORGANS OF INTERNAL SECRETION OF DOGS OF LITTER 14

Although all but one of animals of this litter were sick at time of death, some information can be obtained from the microscopic examination of the organs.

Pancreatic glands of thymectomized dogs 71 and 75 showed extensive infiltration of interstitial tissue with leukocytes which had gone on to abscess formation in case of dog 71. Otherwise, no differences noted in pancreatic glands of thymectomized and control dogs.

Spleens of dogs 71, 74 and 75 atrophic, those of 71 and 75 exhibiting extreme degrees of atrophy. Spleen of control dog 70-75 moderately atrophic and spleen of control 72-73-74 approximately normal. Malpighian bodies in two last mentioned animals could just be made out microscopically, but in others scarcely with aid of microscope.

Suprarenals.—Width of medulla of suprarenals apparently varied considerably. Sections in which medulla was widest came from dogs 71, 72 and female control. Medulla in male control narrow. Apparently width of medulla varied very considerably in different parts of the same gland. In suprarenal of dog 72, for example, medulla was comparatively wide where cross section of gland was round or triangular, but represented by mere line where cross section was long and thin. Impossible to make any statement in regard to finer structure of suprarenals of dogs of this litter on account of postmortem changes. No essential differences could be discovered, however, in cortices of thymectomized and control dogs.

TABLE 24.—Body Weights and Weights of Organs of Litter 14

	Body Weight, Gm.	Heart, Gm.	Spleen, Gm.	Liver, Gm.	Kidneys, Gm.	Suprarenals, Gm.	Pancreas, Gm.	Testes, Gm.	Thyroids, Gm.	Thymus Gm.
Dog 71.....	1,330	14.0	2.1	74.0	15.3	0.3	6.1	0.2	0.3	....
Dog 75.....	980	10.8	1.01	51.5	19.5	0.4	4.9	0.2	0.2	....
71-75 C.....	1,507	12.8	3.2	107.0	19.7	0.23	7.3	0.3	0.3	0.35
Dog 72.....	1,481	15.7	1.7	100.0	19.8	0.4	5.6	...	0.3	....
Dog 73.....	1,191	12.0	2.1	67.0	9.5	0.29	4.2	...	0.2	....
Dog 74.....	1,200	12.51	2.2	70.8	18.6	0.31	3.3	...	0.12	....
72-73-74 C.....	2,610	19.0	4.8	213.0	25.3	0.6	10.9	...	0.6	0.35

Hypophyses, parathyroids, ovaries and testes of thymectomized dogs not marked by any peculiarities as compared with corresponding organs from control dogs.

Thyroids of both control dogs and of dogs 71 and 74 are normal types. Thyroids of dogs 75 and 72 present on microscopic examination areas with more or less solid appearance. Intermingled with these areas are others in which alveoli are large. In section from dog 72 larger alveoli filled with colloid, but in other parts of section colloid almost altogether absent. In sections of thyroids from dogs 72 and 75 many of alveoli have branched or forked character. No infolding. Thyroid of dog 73 shows so much postmortem degeneration as to be useless for study.

Tissue removed from the thymectomized dogs of this litter for examination for thymus rests were not cut serially. Single sections failed to show presence of any thymus rests.

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